GLOBAL WATER PATHOGEN PROJECT
PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

ANTIMICROBAL RESISTANCE: FECAL SANITATION STRATEGIES FOR COMBATTING A GLOBAL PUBLIC HEALTH THREAT

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Summary

Over the past decades, the growing number of deaths due to antimicrobial resistant infections is beginning to rival those from traditional water, sanitation and health (WaSH) related diseases, such as diarrhea. Environmental pathways associated with water and sanitation systems are an important dimension of the global effort to control antimicrobial resistance (AMR). Yet, as discussed in other chapters, control of enteric pathogens should remain the primary focus of any sanitation system. Here, we describe the global occurrence of AMR bacteria within human and animal excreta, environmental amplification and fate of AMR bacteria within sanitation systems, and techniques for the assessment of AMR. Antibiotic resistance genes (ARGs) may be passed on and taken up by virtually all bacteria, via free DNA (transformation), bacteriophage infection (transduction) and cell-to-cell transfer (conjugation); with most acute concern when in association with infectious pathogens. No accepted AMR target for environmental monitoring is in routine use, but various promising 'AMR indicator targets' are discussed, including extended-spectrum beta-lactamase *E. coli* and an important mobile genetic element used by bacteria for ARG uptake, the class 1 integron. In general, treatment reduction of AMR follows reduction of bacterial pathogens, yet often to a lesser degree. This leads to the potential for ARGs to spread more broadly across bacterial species within environmental niches. Hence, it is important to reduce general loads of bacteria, co-selecting chemical stressors (e.g. antibiotics, biocides), ARGs, and mobile genetic elements in final products, not just pathogens, to reduce the potential uptake and spread of AMR.

1.0 The Problem of Antimicrobial Resistance and the Role of Sanitation

Antimicrobial resistance (AMR) is one of the greatest human health challenges of our time, and is predicted to result in more deaths than those from diarrheal illnesses within the next ten years (WHO, 2016) and may become the leading cause of death by 2050 (O'Neill, 2016a). "Antimicrobials" is a broad term encompassing any agent that kills or inhibits microbes, including bacteria, viruses, and parasites. Some antimicrobials; including heavy metals, quaternary ammonium compounds, and other sanitizers, may be used topically or for general disinfection and hygiene purposes, whereas others are formulated specifically as pharmaceuticals. "Antibiotics" are a subset of antimicrobial pharmaceuticals that specifically kill or inhibit bacteria and traditionally indicates natural compounds, although the term also commonly is meant to indicate synthetic forms as well. Antibiotics, in particular, have come to be relied upon globally as critical life-saving drugs that cure deadly bacterial infections. A wide range of classes of antibiotics have been developed and marketed since penicillin was first discovered in the 1920s, ranging from broad-spectrum antibiotics that target various classes of Gram-negative and Gram-positive bacteria, to narrow-spectrum, which ideally target only the pathogen of interest. Resistance occurs when bacteria develop mutations and/or share their antibiotic resistance genes (ARGs) with other bacteria through a process called horizontal gene transfer (HGT). Bacteria that carry ARGs are better able to survive antibiotic therapy, while their non-ARG competitors are diminished. This makes antibiotic treatment a double-edged sword in which it can provide a vital cure for bacterial illnesses, while use, overuse, and misuse contributes to increasing rates of antibiotic resistance and failure of these drugs to work. Compounding the issue of AMR is that virulence factors are often associated with ARGs and transferred together via HGT (Giraud et al., 2017). Generally, antibiotic resistance has been observed to emerge in pathogenic bacteria within a few years of new antibiotics being released onto the market, with resistance rates steadily climbing. Table 1 provides key examples of bacterial pathogens and antibiotic resistance trends. Several countries and global entities, such as the World Health Organization (WHO), monitor antibiotic resistance trends in the clinical setting. A list of relevant surveillance programs and databases (as of December 2016) is provided in Table A.1.

Table 1. Examples of bacterial pathogens and current antibiotic resistance rates associated with human infections in various parts of the world (CDDEP, 2017)[a]

Pathogen /Country	**Resistant Rates (year) by antimicrobial**							
	FQ[b]	CEPH[c]	AG[d]	CAR[e]	VAN[f]	OXA[g]	AP[h]	GEN[i]
Escherichia coli								
Australia	13% (2015)	11% (2015)	8% (2013)	0% (2015)	NR	NR	55% (2015)	NR
India	78% (2015)	78% (2015)	26% (2015)	15% (2015)	NR	NR	88% (2015)	NR
South Africa	28% (2016)	23% (2016)	17% (2016)	0% (2016)	NR	NR	82% (2016)	NR
UK	16% (2015)	12% (2015)	11% (2015)	0% (2015)	NR	NR	66% (2015)	10% (2014)
USA	29% (2014)	12% (2014)	14% (2012)	1% (2014)	NR	NR	45% (2012)	NR

Pathogen /Country	**Resistant Rates (year) by antimicrobial**							
	FQ[b]	CEPH[c]	AG[d]	CAR[e]	VAN[f]	OXA[g]	AP[h]	GEN[i]
Klebsiella pneumonia								
Australia	4% (2015)	6% (2015)	4% (2015)	0% (2015)	NR	NR	NR	NR
India	71% (2014)	87% (2014)	63% (2014)	56% (2014)	NR	NR	NR	NR
South Africa	36% (2016)	65% (2016)	55% (2016)	7% (2016)	NR	NR	NR	NR
UK	14% (2015)	12% (2015)	10% (2015)	0% (2015)	NR	NR	NR	6% (2014)
USA	14% (2012)	17% (2014)	11% (2012)	6% (2012)	NR	NR	NR	NR
Staphylococcus aureus								
Australia	NR	NR	NR	NR	0% (2015)	18% (2015)	NR	NR
India	85% (2014)	NR	46% (2012)	NR	1% (2015)	39% (2015)	94% (2014)	NR
South Africa	NR	NR	NR	NR	0% (2014)	27% (2016)	NR	NR
UK	NR	NR	NR	NR	NR	11% (2015)	NR	NR
USA	43% (2012)	NR	NR	NR	0% (2012)	43% (2014)	NR	NR
Enterococcus faecalis								
Australia	NR	NR	28% (2015)	NR	1% (2015)	NR	0% (2015)	NR
India	89%	NR	NR	NR	7% (2015)	NR	49% (2015)	NR
South Africa	NR	NR	50% (2014)	NR	1% (2016)	NR	12% (2016)	74% (2014)
UK	NR	NR	31% (2013)	NR	4% (2015)	NR	35% (2014)	55% (2013)
USA	NR	NR	34% (2012)	NR	6% (2014)	NR	1% (2012)	13% (2012)
Enterococcus faecium								
Australia	NR	NR	59% (2015)	NR	50% (2015)	NR	87% (2015)	NR
India	97% (2014)	NR	NR	NR	30% (2015)	NR	87% (2015)	79% (2014)
South Africa	NR	NR	74% (2014)	NR	5% (2016)	NR	96% (2016)	50% (2014)
UK	NR	NR	55% (2013)	NR	17% (2015)	NR	82% (2014)	31% (2013)
USA	NR	NR	13% (2012)	NR	78% (2014)	NR	87% (2012)	34% (2012)

[a]Table 1 data are drawn from ResistanceMap, a tracking tool developed by Center for Disease Dynamics, Economics, and Policy (CDDEP) that summarizes clinical data from multiple global surveillance databases to inform on resistance trends (CDDEP, 2017). [b]FQ- Fluoroquinolones; [c]CEPH- Cephalosporins; [d]AG- Aminoglycocide; [e]CAR- Carbapenems; [f]VAN- Vancomycin; [g]OXA- Oxacillin; [h]AP- Aminopenicillins; [i]GEN- Gentamycin; NR- not reported.

1.1 Global Distribution of Antibiotic Resistance Genes (ARGs)

To date, there is no global surveillance database for monitoring trends in environmental ARGs. This is, in part, the result of the constant stream of newly discovered genes, but also reflects the inability to conduct molecular analyses in many areas of the world. There are, however, numerous reviews and case studies reporting ARG incidence within clinical isolates (e.g., (Poirel et al., 2005;

Kazmierczak et al., 2016). For example, Kazmierczak et al. (2016) reported on a global survey of metallo-beta-lactamase (MBL)-encoding genes among carbapenem-resistant bacteria isolated from clinical samples from 40 countries (2012-2014). The distribution of NDM-, VIM-, IMP-, SPM-type MBL enzymes was 44.2%, 39.3%, 16.5%, and 0% among MBL-positive *Enterobacteriaceae*. In contrast, the distribution of NDM-, VIM-, IMP-, SPM-type MBL enzymes was 1.0%, 87.7%, 11.3%, and 0% among MBL-positive *Pseudomonas aeruginosa*. The authors report geographic variations in prevalence as well, with NDM-types more common in the Balkans, Middle East, and Africa; VIM-types more common in Europe and Latin America; and, IMP-types more common in Asia-Pacific. To date, no MBL-positive isolates have been detected in Ireland, Denmark, Netherlands, Sweden, or Israel. Given the rapidly changing scene in AMR detected in various countries, Table 1 simply provides a snapshot of antibiotic resistance rates associated with human infections across a range of regions. Overall, there is a growing pattern of novel AMR pathogens first reported in a single country, with varying rates (rapid or slow) of transfer by human/food carriers to other parts of the world. With respect to environmental surveillance, the WHO, EU, and selected countries in Asia and Africa have initiated a pilot program that targets extended-spectrum beta lactamase (ESBL)-producing *Escherichia coli* screened from routinely cultured *E. coli* identified in water quality studies (Matheu et al., 2017).

The rise of antibiotic resistance has become a well-recognized global public health threat, with several countries and international bodies beginning to maintain surveillance databases (Table A1) and develop strategies for combatting its spread (WHO, 2014; Office of the President, 2015; O'Neill, 2016b). In particular, global organizations, such as the WHO, have emphasized the need for concerted and coordinated efforts aimed at surveillance that include environmental pathways (WHO, 2015). Such surveillance can aid in our understanding of the main causes of resistance and identify management options to limit its spread across international borders, particularly via travel, import/export of food products, and movement of people and their excreta. Of particular concern are sub-lethal doses given to humans and animals that select for resistant strains (Andersson and Hughes, 2014) and residual antimicrobials and ARGs that are released into the environment (Grenni et al., 2018). Hence, the European Union has taken one key step by banning the use of antibiotics in livestock for purposes other than direct disease treatment, though it is clear that such bans alone will not stop the spread of antibiotic resistance (Kalmokoff et al., 2011; Marshall and Levy, 2011; Massow and Ebner, 2013; Bondarczuk et al., 2016; Di Cesare et al., 2016). In particular, enforcement of policy, offering viable alternatives to antibiotics, and identifying practices to prevent livestock illness in the first place are key to reducing antimicrobial use. Here we emphasize the need to consider strategies to contain the spread of AMR that are synergistic with other general environmental and pathogen reduction benefits when developing and implementing sanitation technologies, which in many regions may also include animal manures.

1.2 Types of ARGs of Concern

Tables 2 to 5 summarize ARGs of clinical concern to last-resort antibiotics; however, the evolution of antibiotic resistance is dynamic and this list is by no means exhaustive. The advantage of targeting these genes is that as they may raise a red flag of direct concern to human health, as treatment failure is more likely when pathogens carry these types of resistance. Overall, each of the ARGs corresponding to the WHO (2017) list of AMR bacteria of medium to high concern have been reported in human/animal excreta. Therefore, the environmental release of these genes may provide an effective pathway of transmission unless adequate sanitary management is in place (see Table 4 for a summary of treatment efficacies).

Table 2. Clinically-relevant ARGs corresponding to last-resort antibiotics and possible targets associated with sanitation systems and animal excreta

Antibiotic	ARG/ target	Gene Target	Location	Associated bacteria/pathogen
		Clinically-relevant		
Cephalosporin	*amp*C	Encode beta lactamase enzymes that hydrolyze and break the beta lactam ring structure	Transmissible plasmids	*Escherichia coli, Klebsiella pneumoniae; Acinetobacter baumannii*
			Chromosomal	*Enterobacteriaceae*
Methicillin	*mec*A	Encodes the low-affinity penicillin-binding protein PBP 2A, which allows continued cell wall synthesis by transpeptidases	chromosomal SCCmec mobile genetic element	*Staphylococcus aureus*

Antibiotic	ARG/ target	Gene Target	Location	Associated bacteria/pathogen
Extended spectrum beta lactamase (ESBL)	*CTX*, *TEM*, *SHV*, *amp*C, *OXA*	Encode beta-lactamase enzymes that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain.	Plasmid	*Enterobacteriaceae*
Carbapenem-resistant beta lactamase	*KPC*, *SIM*	Encode beta lactamase enzymes that hydrolyze carbapenems	Plasmid, chromosome	*Pseudomonas aeruginosa* and *Acinetobacter* spp.; *Enterobacteriaceae*
Metallo-beta lactamase	*NDM* (New Delhi Metallo-beta-lactamase), *IMP*, *VIM*, *SPM*	Encode beta-lactamase enzymes called carbapenemases	Plasmid, chromosome (stability varies)	*Enterobacteriaceae, Acinetobacter baumannii, Shigella boydii, Vibrio cholerae, Aeromonas caviae, Klebsiella pneumoniae*, and *Escherichia coli*
Polymyxin (bacitracin, colistin)	*mcr*-1, *pmr*AB	Target modification	Plasmid (mcr-1)	*Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*, and *Escherichia coli*
Macrolides	*erm*, *msr*		Chromosome plasmid	
Aminoglysides	*aac*, *npm*, *rmt*, *arm*	bacterial rRNA methylation, defect of cellular permeability, and active efflux pumps	Chromosome plasmid transposon	Broad range, Gram-negative and Gram-positive bacteria
Quinolone	*qnr*, *gyr*, *aac* (6′)-Ib-cr,	DNA gyrase and efflux pumps	Chromosome plasmid	*Acinetobacter baumannii, Aeromonas, Citrobacter, Shewanella* spp., and *Enterobacteriaceae* (*E. coli*, *Salmonella*)
Vancomycin	*van*A, B, C	Encodes peptidoglycan precursors (involved in cell wall synthesis) with low affinity for binding vancomycin	Chromosome plasmid	*Enterococcus faecalis, Enterococcus faecium,* and *Staphylococcus aureus*

Table 3. Possible indicators of ARGs for sanitation systems and in agriculture

Antibiotic	ARG/ target	Gene Target	Location	Associated bacteria/pathogen	Reference
Possible indicator ARGs for sanitation systems and in agriculture					
tetracyclines	*tet*	Various	Chromosome plasmid		Chee-Sanford et al., 2009; Borjesson et al., 2010; McKinney et al., 2010; Storteboom et al., 2010
sulfonamide	*sul*		Chromosome plasmid		Vaz-Moreira et al., 2016
erythromycin	*erm*		Chromosome plasmid		Ben Said et al., 2015

Table 4. Possible aquaculture ARG indicators

Antibiotic	ARG/ target	Gene Target	Location	Associated bacteria/pathogen	Reference
Aquaculture Indicator ARGs					
Tetracycline (most common)	*Tet*A, B, D,E, G, H, L, M, O, Q, S, W, 34, 35	proton-dependent efflux pumps or via ribosomal protection	Tn1721, Tn5706, transposons,mobile plasmids, integrons (Class 1,2,3)	*Aeromonas*, *Clostridium*, *Edwardsiella*, *Salmonella*, *Vibrio* spp., *Listeria monocytogenes*, and *E. coli*	Jacobs and Chenia, 2007; Miranda et al., 2013; Done and Halden, 2015
Quinolones	*qnr*A,B,D,S aac(6')-lb-cr, aac(69)-lb-cr, *qep*A, *oqx*AB efflux pumps	DNA gyrase and topoisomerase IV	plasmids	*Aeromonas*, *Edwardsiella*, *Photobacterium*, *Vibrio* spp., and *E. coli*,	Miranda et al., 2013
Phenicols	*flo*R	Encodes efflux protein for florfenicol	IncA/C plasmid	*Aeromonas*, and *Edwardsiella* spp.	Miranda et al., 2013
Sulfonamides	*sul*1, *sul*2		Plasmids, integrons (Class 1)	*Acinetobacter*, *Enterobacteriaceae*, and *Bacillus* spp.	Jacobs and Chenia, 2007; Miranda et al., 2013; Done and Halden, 2015

Table 5. Indicators of gene capture, mobility, and horizontal transfer of ARGs

Antibiotic	ARG/ target	Gene Target	Location	Associated bacteria/pathogen	Reference
Horizontal Gene Transfer					
Class 1 integron	intI1	integrase enzyme	Chromosome plasmid	Associated with resistance genes for fluoroquinolones, trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, piperacillin/tazobactam in many genera, and multidrug-resistance *E. coli*	Kotlarska et al., 2015; Aubertheau et al., 2017
Incompatibility plasmid	incI1, incl2	Typically carry multiple ARGs	plasmids	Enterobacteriaceae	Dropa et al., 2016; Ovejero et al., 2017
transposons	transposon	contain integrons—more complex transposons contain a site for integrating different ARGs and other gene cassettes in tandem for expression from a single promoter	Chromosome plasmid Naked DNA	Gram-positive and Gram-negative bacteria	Levy and Marshall, 2004
Broad host range plasmids	Wide range ARG	multiple	plasmid	Wide range of bacteria	Akiyama et al., 2010; Jain and Srivastava, 2013

1.3 Transmission of ARGs

Sanitation is a logical critical control point to aid in reducing the spread of antibiotic resistance. Human and animal waste-streams contain antibiotic resistant bacteria (ARBs), ARGs, antibiotics, metals, and other potential agents that could exert selection pressure for AMR. Depending on how the waste is treated and handled, resistance levels can increase or decrease (Marti et al., 2013; Larson, 2015; Bengtsson-Palme et al., 2016; Bondarczuk et al., 2016; Holman et al., 2016; Qian et al., 2016). Ideally, sanitation technologies can be adapted to serve their intended purpose of minimizing human exposure to fecal pathogens, while also reducing the potential spread of ARGs to human pathogens or to the reservoir of resistance in the natural human, aquatic, and soil microbiomes. However, in order to synergistically achieve these goals, it is critical to understand the nature of risk posed by antibiotic resistance and how it differs from pathogens and fecal indicators that have traditionally served as treatment targets.

Figure 1 illustrates how fecal contamination pathways in the environment may also serve as dissemination routes for the spread of AMR. The spread of AMR is distinct, however, as the DNA that confers resistance (i.e., ARGs) can be spread among different (including non-pathogenic) species of bacteria by HGT mechanisms, including conjugation (mating between bacteria) and transduction (via bacteriophage infection). ARGs from dead organisms, existing as free DNA in the environment, may also be assimilated by downstream bacteria by a process called transformation – hence disinfection of excreta alone may not be totally effective at preventing the spread of AMR. Also, natural and engineered stressors (such as disinfectants and disinfection by-products (Zhang et al., 2017) can induce mutations and select for biocide resistance and co-select for ARB leading to AMR (Baharoglu et al., 2013; Culyba et al., 2015). Sanitation technologies should ideally aim to reduce the conditions for selection and HGT (including to clinical strains) of ARGs and to physically destroy ARGs where possible (Bouki et al., 2013; Al-Jassim et al., 2015; Bengtsson-Palme et al., 2016). Mixing pathogenic bacteria within environments containing high densities of active bacteria and in the presence of selective and stress agents, such as antibiotics and metals, may increase the potential for horizontal transfer of ARGs (Abraham, 2011; Andam and Gogarten, 2011). Mixing of waste streams with high concentrations of antibiotics, such as from pharmaceutical manufacturing facilities or feedlot manures where sub-therapeutic concentrations of antimicrobials are used, with those containing human pathogens, such as domestic waste, is not recommended (Sidrach-Cardona et al., 2014). Segregated treatment of hospital waste has also been suggested as a “hot spot” control strategy (Rodriguez-Mozaz et al., 2015).

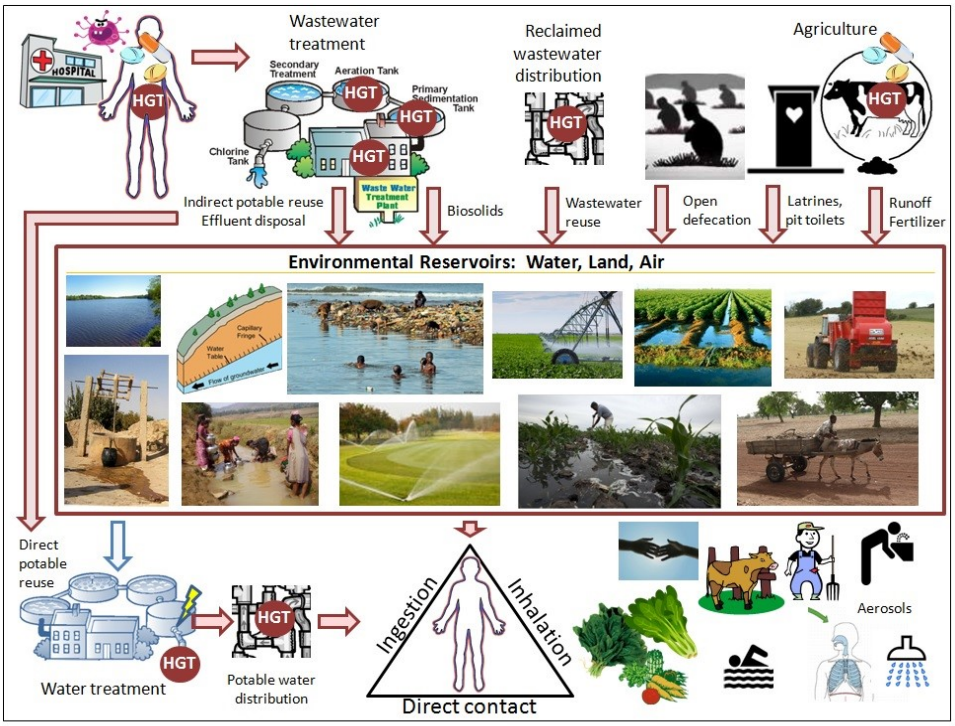

Figure 1. Environmental pathways of AMR showing sanitation as critical control points (red arrows) for dissemination of ARBs and ARGs. Also highlighted are likely hotspots for horizontal gene transfer (HGT). Environmental reservoirs include drinking water sources (groundwater, shallow wells, surface water), recreation/bathing water sources, irrigation (crop, turf), and biosolid/compost/manure storage or land application

1.4 Risk assessment for AMR

An important avenue for focused scientific effort is in the development of human health risk assessment models specifically tailored to antibiotic resistance. Microbial risk assessment, including quantitative microbial risk assessment (QMRA), serves to estimate the probability of human infection, given a defined exposure dose and exposure route(s) (Ashbolt et al., 2013). However, new models are needed that consider HGT and the fact that resistant infection following exposure may not be immediate. For example, elevating resistance levels among non-pathogenic environmental bacteria (e.g., through ineffective sanitation measures or those using high microbial activity) could increase the probability of transferring ARGs to native bacteria and human pathogens in the environment (especially if waste streams are mixed) or potentially to pathogens on human skin or within the gut microbiota itself. The ultimate "risk" then is defined not just as an infection itself, but as failure of antibiotics to cure an infection, or "treatment failure".

Developing risk models with the goal of informing the management of antimicrobial resistance will take time and will require elements of dynamic disease transmission modeling not traditionally used in QMRA. Thus, we are wise to proceed in parallel with the advancement of mitigation technologies that conservatively target both pathogen and ARG reduction and ideally are low-cost and work within the framework of existing sanitation goals (Pruden et al., 2013).

2.0 Environmental Occurrence and Persistence

Environmental and clinical reservoirs of resistance are linked and employ conditions that exert selection pressure, or that are conducive to HGT and exacerbate the spread of antibiotic resistance. A significant body of scientific literature has grown in the last decade, documenting how human activities along with animal manure management can serve to increase background levels of resistance in soil and water environments (Singer et al., 2006; Cantas et al., 2013; Rizzo et al., 2013b; Blaak et al., 2015a; Sharma et al., 2016; Singer et al., 2016; Zhu et al., 2017). Together, there is substantial evidence that environmental routes of resistance dissemination can contribute to evolution of resistant pathogens that ultimately appear in clinics and hospitals (Taylor et al., 2011; Hölzel et al., 2012; Ma et al., 2016a).

In practice, it can (fortunately) be difficult to detect clinically-relevant genes in environmental matrices, which can make them poor targets for certain applications, such as assessing the likely benefits of various sanitation technologies for mitigating the spread of ARGs (Table 2). For this reason, more commonly detected genes in the environment, such as the sulfonamide and tetracycline ARGs, are popular among researchers (Bengtsson-Palme et al., 2016; Pei et al., 2016). While resistance to these antibiotics is rarely a serious clinical concern because their corresponding resistance determinants have become widespread, they can provide informative targets for predicting how ARGs may respond to treatments or behave in the environment. For example, Pruden et al. (2012) reported a near perfect correlation between the *sul*1 sulfonamide ARG and upstream densities of livestock operations and wastewater treatment plants. Therefore, such commonly occurring genes may serve as "AMR indicator genes". HGT markers or determinants (Table 5), are not technically ARGs, but are considered to be indicative of the potential for ARGs to be transferred among bacteria, which is arguably the ultimate concern (Gillings, 2014; Culyba et al., 2015; Sharma et al., 2016). If ARGs stay confined within a non-pathogenic host, then this is not as much of a concern as if they are transferred, or have the potential to be transferred, to a pathogen. Targets include gene markers for plasmids, particularly the highly transferrable plasmids such as those within certain incompatibility "inc" groups, integrons, transposons and other mobile genetic elements, all of which have been noted in some cases to carry several ARGs (Chang et al., 2016; Folster et al., 2016; Saito et al., 2016).

Recently it was reported that, similarly to *sul*1, the *intI*1 gene encoding class 1 integrase is a strong indicator of "pollution" (Gillings et al., 2015), including resistance to fluoroquinolones, trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, piperacillin/tazobactam, and presence of multidrug-resistance *E. coli* (Kotlarska et al., 2015). Also, the European COST Action group recommended a strategy of monitoring a mixture of clinical ARGs, indicator ARGs, and gene transfer markers, and an international cross-comparative study led by the NORMAN network that is currently underway (Berendonk et al., 2015; COST, 2017; NORMAN, 2017).

2.1 Detection Methods for Antibiotic Resistance Monitoring Targets

Special consideration is needed for the monitoring of antibiotic resistance, particularly for assessing the effectiveness of sanitation technologies and tracking any significant change in the spread of resistance via environmental routes. Monitoring methods largely fall into two classes: 1) culture-based methods and 2) molecular methods. The pros and cons of these methods for tracking antibiotic resistance in the environment have been extensively reviewed (Luby et al., 2016; McLain et al., 2016). Here we provide a brief overview and highlight some key points in the context of local sanitation systems.

When monitoring for AMR it is critical to recognize that, just as antibiotics are largely natural or naturally-derived compounds, there is a ubiquitous background level of

antibiotic resistance for certain ARGs (Rothrock et al., 2016). Microbes have evolved the ability to both produce antibiotics (e.g., to ward off competitors), as well as the ability to resist antibiotics (Davies, 2006; Martinez, 2008; Forsberg et al., 2012; Culyba et al., 2015; Westhoff et al., 2017). While it is true that antimicrobial resistance is a natural phenomenon, what has changed in the modern era are the sheer concentrations and loadings of antibiotics and other selective agents to which microbes are being exposed. Elevated levels of antibiotics are a direct result of mass industrial production, use in humans, companion animals and livestock, and corresponding release and excretion into the environment. Thus, ideally, culture-based and molecular-based monitoring technologies are designed to identify changes in the kinds and levels of these resistance indicators against a relevant background.

In terms of culture-based techniques, some consensus is emerging around *E. coli* as a highly suitable target (Blaak et al., 2015b; Liang et al., 2015), although many other potentially useful bacterial targets, such as *Klebsiella spp.* (Berendonk et al., 2015), fecal enterococci (Berendonk et al., 2015), and bacteria that grow in aquatic/soil environments such as *Pseudomonas aeruginosa* (Santoro et al., 2015) or various *Aeromonads* (Varela et al., 2016) exist. However, *E. coli* is a practical choice given that it is already the most widely monitored target as an indicator of fecal pollution and thus methodologies are already standardized and infrastructure is more likely to be in place to implement monitoring campaigns based on *E. coli* (Matheu et al., 2017).

Minimum inhibitory concentrations (MICs) for most antibiotics are largely defined for susceptible *E. coli*, making it relatively straightforward to either incorporate antibiotics into *E. coli* selective media, or perform MIC breakpoint assays on isolated bacteria. The latter can be accomplished in 96 well trays using the Kirby-Bauer disk diffusion assay (Bauer et al., 1966; CLSI, 2015). This enables assessment of antibiotic resistance under defined conditions: using a viable strain phenotypically expressing resistance in a manner that can be directly compared to known MICs. Further advantages are that *E. coli* is generally a fecal-associated organism (thus maintaining relevance to tracking fecally-derived sources of antibiotic resistance) (Ashbolt et al., 2001). Importantly, some *E. coli* strains are known pathogens and many strains are also known to be capable of receiving and transferring genes within or between species (Kotlarska et al., 2015).

There are numerous resistant pathogens of major global concern (WHO, 2017), several of them summarized in Table 1; it is unknown to what extent the behavior of resistant *E. coli* is representative of other resistant pathogens, particularly those that grow well in water/sanitation environments, such as *Aeromonas spp., Arcobacter spp.*, and *P. aeruginosa*. A further general downside of culture-based techniques is that they will not provide information about the broader microbial ecological behavior of ARGs, given that environmental samples will typically contain billions of microbes and their mobile genetic elements, with culture-based techniques capturing only a small fraction. Methods such as heterotrophic plate counts incorporating antibiotics into their culture media can provide insight into the behavior of broader groups of bacteria than group-selective media, but still will capture only culturable bacteria, a tiny fraction of the true bacterial community (Bartram et al., 2004). All will suffer from not knowing the identities of the isolated bacteria and thus not being able to differentiate acquired resistance from intrinsic resistance (Cox and Wright, 2013; e.g., a Gram-positive organism growing in the presence of an antibiotic targeting Gram-negatives is “intrinsically resistant”). Also, culture-based methods are generally extremely laborious and time-consuming and thus not ideally suited for extensive monitoring or certain research applications.

Molecular-based methods present the advantage of directly targeting ARGs as the presumptive agents of resistance while also circumventing biases associated with culture-based techniques. However, the simple presence of a gene does not mean it is functional or capable of being expressed. ARGs can be transferred horizontally, thus transcending their bacterial hosts. Further, given that they strongly correlate with anthropogenic inputs (Gaze et al., 2011; Pruden et al., 2013; Rizzo et al., 2013b; Ahammad et al., 2014; Graham et al., 2014; Singer et al., 2016), ARGs have been described as “pollutants” in their own right (Pruden et al., 2006). In Table 6 several available molecular methods for antibiotic resistance monitoring are summarized. Just as there are tens of thousands of species of bacteria in an environmental sample, there appears to be thousands of different types of detectable ARGs. A potential problem in only targeting ARGs via molecular methods is that such genes may not be expressed and/or passed on to pathogens of concern. Expression in cultured isolates provides more definitive information on functionality of ARGs within a viable host (Wichmann et al., 2014; Ma et al., 2016a; Bengtsson-Palme et al., 2017; Surette and Wright, 2017; Zhu et al., 2017). This brings to question, which ARGs and/or mobile genetic elements to monitor?

As indicated in Table 6, there are several available methods including qPCR and numerous other assays which are used for ARG targets. In general, there are three categories of relevant gene targets: 1) ARGs of direct clinical concern; 2) indicator ARGs; and 3) determinants for gene mobilization. ARGs of clinical concern include those encoding resistance to last-resort antibiotics, such as vancomycin, carbapenems or colistins (Hocquet et al., 2016; Mediavilla et al., 2016; Sharma et al., 2016; EFSA, 2017; Al-Tawfig et al., 2017).

Table 6. Molecular methods for antimicrobial resistance monitoring

Method	Target/Units	Units	Advantages	Disadvantages	References
PCR	Specific DNA sequence (gene)	Presence/ absence	Robust, well documented	Highly specific; must have known ARG sequence and primers Semi-quantitative	Sung et al., 2014
q-PCR	Specific gene	Gene copies per mass or gene copies/16S gene copies	Robust, well documented Quantitative	Must have known ARG sequence and primers Multiplexing is challenging May need probes to improve specificity Equipment and reagent costs ~conventional PCR given high throughput	Hu et al., 2017; Narciso-da-Rocha and Manaia, 2017
q-PCR array	Hundreds of genes per array	Gene copies per mass or gene copies/16S gene copies	Quantitative for multiple genes	Limited to known ARG sequence and primers Specificity and detection limit difficult to verify Equipment costs much greater than conventional PCR	Xie et al., 2016
Metagenomic sequencing	All DNA, depth reflects number of total sequences	Relative abundance of ARGs (ARG sequences per total sequences) or ARG percentage (ARG type per total housekeeping gene sequences)	Captures full range of resistance elements without selecting targets a priori Sensitivity is directly related to the number of sequences returned (depth)	Difficult to confirm that a true ARG has been detected. Not quantitative. Available databases for comparison are limited, but rapidly growing. Expensive (~4K for shallow sequencing of ~10 samples)	Ju and Zhang, 2015; Bengtsson-Palme et al., 2016; Munk et al., 2017
Functional Metagenomics	Gene	Presence/absence	Can discover new ARGs. Verifies functionality of ARG.	Highly tedious and labor-intensive. A great deal of effort can be expended to discover 1 new ARG.	Bengtsson-Palme et al., 2014; dos Santos et al., 2017

Ideally, all ARGs would be monitored, both in terms of types present, their relative abundances, their propensity to be horizontally transferred (i.e., occurring on a mobile genetic element such as a plasmid or transposon), and the types of bacterial hosts in which they are present. This is precisely what is sought to be achieved via the new and emerging field of metagenomics (Pal et al., 2016). Through application of next-generation DNA sequencing technology (e.g., Illumina sequencing, pyrosequencing) and most recently third-generation DNA sequencing technology (e.g., PacBio or MinION), DNA extracted from environmental samples can be fragmented and directly sequenced. Through bioinformatics pipelines, the DNA reads can then be compared against available databases of known ARGs, such as RESFINDER for BLAST analysis (Zankari et al., 2012; Zankari et al., 2013), MGMAPPER for mapping of reads (https://cge.cbs.dtu.dk/services/MGmapper/)(Petersen et al., 2017), and MEGARes (Lakin et al., 2017), Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) (Gupta et al., 2014), the Antimicrobial Resistance Database Project (Liu and Pop, 2009), the Comprehensive Antimicrobial Resistance Database (McArthur et al., 2013; Jia et al., 2017), the Structured Antibiotic Resistance Gene Database (ARGs-OAP; (Yang et al., 2016) or deepARG (Arango-Argoty et al., 2017). In this manner, a profile of the types and numbers of ARGs detected in a sample can be obtained and compared with other samples using various bioinformatics techniques and graphical representations. Metagenomics has been successfully applied in this manner for monitoring ARGs in wastewater treatment plants (Schluter et al., 2008; Wang et al., 2013; Yang et al., 2014; Li et al., 2015a; Munck et al., 2015; Zhang et al., 2015a; Bengtsson-Palme et al., 2016; Hu et al., 2016; Karkman et al., 2016; Ma et al., 2016b), biosolids (McCall et al., 2016; Tao et al., 2016; Rowe et al., 2016; Tang et al., 2016), manure (Agga et al., 2015), soil (Yan et al., 2016), rivers (Garner et al., 2016; Rowe et al., 2016), sediments (Cummings et al., 2011), and

estuaries (Port et al., 2012). However, next-generation DNA sequencing technologies are still costly and require a high level of expertise, currently restricting metagenomic analysis to the realm of research, although that may soon change.

To cut costs, metagenomic studies often sequence multiple samples per lane, with a shallow sequencing approach multiplexing ten wastewater activated sludge samples per Illumina lane or flow cell and successfully being able to detect and compare ARG profiles (Cai and Zhang, 2013). However, deep sequencing (e.g., one sample per Illumina lane), which is even more costly, may be required to filter out dominant and housekeeping genes and identify rare ARGs of interest. Deep sequencing is also typically necessary to link ARGs with host bacteria and genetic elements, along with sophisticated genome assembly techniques, which require a high level of expertise, are not standardized, and still error prone. DNA sequencing costs are predicted to decrease significantly in the coming years and new user-friendly technologies are currently in the pipeline (Schmidt et al., 2017). In particular, third-generation DNA technologies reduce cost and produce longer reads, which will facilitate assembly and thus identifying which hosts carry ARGs and if they are associated with mobile genetic elements. Thus, the metagenomic approach may soon be a widely accessible gold standard for ARG monitoring.

While metagenomic methods are still under development, quantitative polymerase chain reaction (qPCR) has become a well-established tool for monitoring ARG targets of interest. Using one of several available fluorescence-based assays, a real-time PCR instrument, and appropriate standard curve, it is possible to precisely quantify the ARG/determinant of interest. Such quantitation has been of value for quantifying anthropogenic inputs of ARGs to the environment (Pruden et al., 2012; Graham et al., 2016) and assessing the effectiveness of waste treatment technologies (Ma et al., 2011; Narciso-da-Rocha and Manaia, 2017). The disadvantage of qPCR is that, while it is less time-demanding than culture-based techniques, it is realistically only possible to include a handful of ARGs in any monitoring scheme. This necessitates selecting appropriate ARG monitoring targets.

2.2 Environmental Reservoirs

Antibiotic resistance has existed on earth for millennia, and evolved with bacteria; thus, it is important to benchmark success of AMR control/mitigation with respect to an appropriate background (Rothrock et al., 2016). Background distributions of various levels of ARGs exist, along with various mechanism described above for their selection and transfer. Therefore, the concern addressed in this chapter with respect to environmental reservoirs of ARGs is the intensification ('hot-spot') development of the resistome and potential for vertical (within the species) or horizontal (between species) gene transfer within the environment (von Wintersdorff et al., 2016) and ultimately to clinically-relevant bacteria associated with sanitation-related technologies.

While evidence for the role of environmental pathways for AMR of clinical relevance exists today (Quintela-Baluja et al., 2015), it has not yet become a high priority for healthcare professionals. This apparent lack of awareness has also impacted on financing studies to clarify the role of the environment, with few funded projects thus far focusing on food, livestock wastes, and companion animals (e.g., EU Project EFFORT, http://www.effort-against-amr.eu/page/activities.php, Songe et al., 2017). Therefore, we present examples of AMR environmental reservoirs that particularly highlight the scientific plausibility/concern should mitigation/reduction approaches not be considered with sanitation systems. While most understanding of AMR is associated with bacteria and their bacteriophages, enteric viral, parasitic protozoan and helminth pathogens could also develop antimicrobial resistance, but are not capable of horizontal gene transfer in the sanitation environment the way that bacteria are. Table 4 provides a comprehensive summary of likely efficacy of AMR reduction by sanitation systems at the time of writing this Chapter. However, it is important to note that some report on a culture-basis while others by molecular methods and information about efficacies of these treatments is evolving.

2.3 Presence and transfer of relevant genes within environmental bacteria

Ample evidence exists for native (autochthonous) bacteria in the environment taking up and maintaining ARGs (Walsh et al., 2011; Cantas et al., 2013), such as vancomycin-resistant *Enterococcus faecium* (VREfm) (Sacramento et al., 2016). Indeed, the study of antibiotic-resistance mechanisms in environmental bacteria is shedding light on novel pathways of resistance found in pathogens (Spanogiannopoulos et al., 2014). Particular concern may come from spore-forming clostridia, given the persistence of their spores in soil systems (Gondim-Porto et al., 2016) and the increasing resistance within pathogens like *Clostridium difficile* (Zaiss et al., 2010; Garner et al., 2015). However, as with a range of bacterial pathogens, non-pathogenic sub-species or clades are likely to exist in the environment that are not only poorly documented, but would confound the relevance of detections, as in this case for *C. difficile* with ARGs in the environment (Janezic et al., 2016). Furthermore, ongoing genetic studies are leading to bacterial reclassifications, with *C. difficile* now assigned to a new genus, *Clostridioides difficile* (Lawson et al., 2016).

Amongst the various determinants associated with ARG capture, uptake and transfer within bacteria (Singer et al., 2016), class 1 integrons (e.g. *intI1*, integrase of class 1 integrons) are often involved (Stalder et al., 2012). Class 1 integrons routinely contain mobile antibiotic and biocide-resistance genes (Stokes and Gillings, 2011) and are described as part of the "mobilome" (Tian et al., 2016). For example, class 1 integrons were validated as a proxy for anthropogenic ARG inputs to the Thames River basin by Amos et al. (2015), who modeled various contributing factors impacting environmental resistome presence and determined that wastewater effluent was the major source. Class 1 integrons may also reflect the history of ARG input to soil, as seen in sludge amended soils (Burch et al., 2014),

which may respond in a similar way to soils impacted by open defecation or applied excreta following a range of treatment options.

Given that ARGs and corresponding bacteria and environments in which they have been identified have been fairly widely surveyed at this point and are quite numerous, here we focus on exemplar scenarios (e.g., worldwide spread of mcr-1 gene resistance within a year (Liakopoulos et al., 2016)) and opportunities to limit the potential enrichment of "hot-spots" for ARG amplification (Pruden et al., 2013). The efficacy of such interventions could then be tracked with respect to the prevalence of AMR surrogates, such as class 1 integrons or other "indicator" ARGs identified in Table 3 (e.g. (Spanogiannopoulos et al., 2014; Blaak et al., 2015a), and minimizing the environmental release of antibiotics, biocides and metals that are known to increase selection for AMR (Di Cesare et al., 2016; Singer et al., 2016).

2.4 Presence and transfer of extracellular genes within the environment

In addition to whole cells containing ARGs, there is a need to consider extracellular ARGs. As only focusing on genes within allochthonous (i.e., introduced) bacteria (or other cellular pathogens) may miss development or release of important ARGs. Hence, in addition to the use of molecular methods to assess the environmental resistome, as described above, we need to consider extracellular ARG uptake, by naked (transformation) and bacteriophage (transduction) mechanisms. While novel gene uptake by transduction is generally considered important (Ross and Topp, 2015), there are mixed views as to the significance of ARGs within environmental bacteriophages on the development of environmental AMR due to misinterpretation from sequence information (Enault et al., 2017) and given the high concentration of active host cells generally needed to provide interactions, as seen in clinical environments (Stanczak-Mrozek et al., 2015). Nonetheless, environmental transduction has been demonstrated (Anand et al., 2016) and the persistence of ARGs is clearly influenced by the greater persistence of bacteriophages in the environment versus ARB (Calero-Cáceres and Muniesa, 2016) or novel superspreaders (Keen et al., 2017). Therefore, sanitation processes that are focused on enteric virus nucleic acid elimination may also be effective in reducing the release and presence of bacteriophage/plasmid-mediated environmental ARGs.

Furthermore, naked DNA uptake of ARGs (transformation) is also possible during or after inactivation of pathogens and their subsequent release of ARGs (genomic or plasmid-borne). For example, advanced oxidation processes generate reactive oxygen species (ROS), which can damage cell membranes and elicit cellular SOS responses. The SOS response has been shown to increase integrase activity and the rate of gene recombination, increase the rate of HGT (Beaber et al., 2004; Guerin et al., 2009; Baharoglu et al., 2012), and increase competence which in turn may promote plasmid transformation in wastewater treatment (Ding et al., 2016). Other environmental stresses (Aertsen and Michiels, 2006), such as heat shock (Layton and Foster, 2005), starvation (Bernier et al., 2013), high hydrostatic pressure (Aertsen et al., 2004), and high pH, as well as the presence of antimicrobials, disinfection chemicals or UV have also been shown to induce the SOS response (Poole, 2012).

2.5 Fate of AMR-related genes within sanitation systems

Given the above general discussion of likely mechanisms for environmental ARG amplification and spread, some guidance is presented below to highlight possible management options to reduce environmental AMR spread via sanitation systems.

2.5.1 Amplification (enrichment) versus reduction

In general, manures and sewage sludge (biosolids) are recognized matrices with the highest concentration of ARGs and antimicrobials, possibly up to 1000-times the concentrations present in wastewater effluents (Munir et al., 2011). Therefore, it is most important to control ARG release from these excreta-related solids. Significant reductions in ARGs are possible via bio-drying sludge (10-15 day process) compared to traditional composting (30-50 days). For example, Zhang et al. (2016a) demonstrated by molecular methods, some 0.4 to 3.1 $\log_{10}$ reductions in ARGs and a similar level of reduction in mobile genetic elements with bio-drying. The success in reductions was related to changes in the microbial communities that developed (microbiomes), which largely reflected physiochemical changes, such as pH, available nutrients, temperature, and moisture content (Zhang et al., 2016a). Hence, manipulation of the microbiome, as also seen in anaerobic digestion and composting (Youngquist et al., 2016), influences the fate of ARGs. With regards to persistent spore-forming bacteria as indicators it seems that the fecal indicator *Clostridium perfringens* may be a conservative indicator for ARG-containing *C. difficile* spores with regards to thermal (composting) treatment (Xu et al., 2016).

A recent review by Youngquist et al. (2016) suggests that mesophilic anaerobic digestion virtually eliminates ARB when assayed using culture-based methods (Beneragama et al., 2013). However, ARGs are readily moved across viable bacteria in the community, most of which are unlikely to be culturable on standard agar plates. This highlights the importance of utilizing direct measures (such as sequence-based resistome or qPCR assays) to detect ARGs. While most of these molecular-based methods fail to discriminate between dead and living targeted cells, quantitative changes can still be followed. For example, Christgen et al. (2015) demonstrated that a combination of anaerobic digestion followed by aerobic polishing provided the most reduction in ARGs identified by sequencing in an evaluation of six different treatment trains for treating domestic wastewater. Nonetheless, while the anaerobic-aerobic sequencing treatment of domestic wastewater effectively reduced aminoglycoside, tetracycline, and β-lactam ARG levels relative to anaerobic units, sulfonamide and chloramphenicol ARG levels were largely unaffected by

any treatment and there was also a general increase in multi-drug resistance presence in all effluents (Christgen et al., 2015). Hence, further treatment or containment of effluent would be necessary to minimize potential AMR issues, as subsequent soil application may not result in effective removal across the range of ARG and their determinants (Burch et al., 2014). Despite the genetic burden in carrying a functional integrase, modeling indicates that the presence of this gene enables a population to respond rapidly to changing selective pressures, so maintenance of class 1 integrons is no surprise (Engelstadter et al., 2016).

In summary, ARG transfer and potential increase within the native microbiota is very likely in any sanitation system where microbial activity is encouraged (such as anaerobic digestion, trickling filters, aerobic reactors, compost, stored urine or wastewater lagoons), and in general, because of the higher solids content including microorganisms, sludge/biosolids/biofilms that support high density growth. Key factors for AMR transfer include selecting factors (antimicrobial, biocide and heavy metal concentrations), biotic processes (biofilm growth, high bacteriophage density, mobile genetic elements, etc.), and certain abiotic conditions (pH, temperature, moisture content, sunlight) that favor microbial activity. Specific issues with different treatment (unit) processes are discussed next.

3.0 Reductions by Sanitation Management

Treatment technologies that provide benefits for inactivating bacterial pathogens and which also may help to minimize the spread of antibiotic resistance.

3.1 Fate of ARGs versus host bacteria by unit processes

Most sanitation processes involve bacterial activity and given the above discussion on inevitable mobilization of ARGs to members of the resident microbial community, we need to focus on actions documented to reduce ARGs or influential mobilome elements, as recently reviewed (Bouki et al., 2013; Rizzo et al., 2013b; Sharma et al., 2016). Common unit processes are now briefly reviewed below so as to give a sense of which issues to consider, in addition to traditional focus on pathogens.

3.1.1 Dry sanitation and collected urine streams

If lime or similar (fly ash) types of alkali compounds are added to dry sanitation systems and the pH exceeds 10, then much of the above discussion on pH and ammonia effects would be expected to be applicable in terms of reducing ARGs occurrence. Desiccation may also be important via inactivation of microbial processes and, in general, 12 months storage time is recommended for pathogen control (Schönning et al., 2007).

For collected urine (yellow water), there is a high likelihood of residual antimicrobial compound presence (i.e., selecting factors), along with antibiotic-resistant urinary tract bacterial pathogens (Ejrnaes, 2011). Hence, minimizing transfer to the highly active bacteria community within separated urine streams is important, but largely an unreported aspect to date (Pynnonen and Tuhkanen, 2014; Bischel et al., 2015). Current pathogen control regulations for source-diverted urine recommend around six months of storage for pathogen inactivation (Höglund et al., 2002; Tilley, 2016); however, reductions in antimicrobials may only be some 42-99% for anti-tuberculosis drugs and < 50% for some antivirals and antibiotics (Jaatinen et al., 2016). Therefore, additional treatments, such as UV alone or in combination with peroxydisulfate, are recommended to further eliminate antimicrobials in collected urine (Zhang et al., 2016b). However, based on the principles described above, the native microbiota within stored urine would be expected to accumulate ARGs, hence soil application or further treatment is recommended to reduce AMR issues.

3.1.2 Wetland/pond sanitation systems

Sediments within sanitation wetland/pond systems and receiving water sediments may be “hot-spots” for AMR development (Cummings et al., 2011), due to increased microbial activity and influx of wastewater-borne ARGs compared to free-waters above. Nonetheless, constructed wetlands have been shown to effectively reduce ARGs ($\log_{10}$ reductions of 0.26-3.3) and antimicrobials (Huang et al., 2015; Chen et al., 2016) and thus could provide a net protective effect prior to effluent reuse applications in agriculture.

Table 7. Lagoons, oxidation ditches, and wetlands

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Outa	Log Removal	Reference
Oxidation ditch + Secondary settling (with partial sludge recycling)	Municipal WWTP using Oxidation ditch as main treatment process. (Data estimated from Figure 2.)	Hefei, China	16S rRNA	1.81E+07[bd]	6.74E+06	0.429	Li et al., 2017
			*int*I1	3.00E+05[bd]	9.00E+04	0.52	
			*sul*1	4.00E+06[bd]	9.00E+04	1.65	
			*sul*2	2.00E+05[bd]	6.00E+04	0.52	
			*tet*O	2.00E+04[bd]	4.00E+03	0.70	
			*tet*Q	1.00E+06[bd]	4.00E+04	1.40	
			*tet*W	3.00E+03[bd]	1.00E+03	0.48	
Oxidation Ditch	Full scale WWTP serving a population of 285,000. WWTP processes include Grit Removal, Oxidation Ditch, and Constructed Wetland. (Data estimated from values reported on Figure 2)	Hangzhou, China	16S rRNA	7.11E+08[bd]	1.74E+07	1.611	Chen and Zhang, 2013
			*int*I1	8.00E+07[bd]	2.00E+06	1.60	
			*sul*1	1.00E+07[bd]	5.00E+05	1.30	
			*sul*2	2.00E+07[bd]	8.00E+05	1.40	
			*tet*M	4.00E+07[bd]	6.00E+04	2.82	
			*tet*O	3.00E+07[bd]	4.00E+04	2.88	
			*tet*Q	8.00E+07[bd]	1.00E+05	2.90	
			*tet*W	6.00E+07[bd]	1.00E+06	1.78	
Oxidation Ditch	Full scale WWTP serving a population of 94,500. Processes include: Grit Removal, Oxidation Ditch and Biological Aerated Filter. (Data estimated from values reported on Figure 2)	Hangzhou, China	16S rRNA	4.28E+09[bd]	4.81E+07	1.949	
			*int*I1	8.00E+07[bd]	2.00E+06	1.60	
			*sul*1	8.00E+07[bd]	3.00E+05	2.43	
			*sul*2	2.00E+08[bd]	3.00E+06	1.82	
			*tet*M	1.00E+07[bd]	5.00E+04	2.30	
			*tet*O	4.00E+06[bd]	1.00E+04	2.60	
			*tet*Q	1.00E+07[bd]	2.00E+04	2.70	
			*tet*W	1.00E+08[bd]	4.00E+05	2.40	
Oxidation ditch (aerobic tank)	Full WWTP receiving domestic sewage from urban and residential areas, serving 300,000 people (Composite samples collected March to May, 2013. Sample concentrations estimated with WebPlotDigitizer from figure S3)	Linan City, China	*int*I1	1.16E+10[bd]	2.88E+09	0.605	Li et al., 2015
			*sul*1	9.09E+09[bd]	4.30E+09	0.325	
			*sul*2	1.67E+09[bd]	7.66E+08	0.338	
			*tet*A	7.78E+08[bd]	8.50E+07	0.962	
			*tet*B	4.14E+07[bd]	4.93E+06	0.924	
			*tet*C	8.60E+08[bd]	1.79E+08	0.682	
			*tet*G	1.51E+09[bd]	3.90E+08	0.588	
			*tet*L	4.85E+07[bd]	1.49E+07	0.513	
			*tet*M	3.43E+08[bd]	6.39E+06	1.730	
			*tet*O	4.24E+09[bd]	6.45E+08	0.818	
			*tet*Q	1.18E+10[bd]	1.25E+09	0.975	
			*tet*W	5.04E+09[bd]	3.43E+08	1.167	
			*tet*X	8.85E+08[bd]	1.67E+08	0.724	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Outa	Log Removal	Reference
Aerobic tank/triple oxidation ditch	Full scale medium sized WWTP capacity 60,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	1.50E+10[bd]	8.00E+08	1.273	Li et al., 2016
			*sul*1	2.00E+10[bd]	1.30E+09	1.187	
			*sul*2	5.00E+06[bd]	5.50E+05	0.959	
			*tet*A	5.00E+09[bd]	4.00E+08	1.097	
			*tet*B	8.00E+07[bd]	8.00E+06	1.000	
			*tet*C	7.00E+10[bd]	3.00E+09	1.368	
			*tet*G	5.00E+09[bd]	8.50E+08	0.770	
			*tet*L	8.00E+07[bd]	9.00E+06	0.949	
			*tet*M	4.50E+08[bd]	2.00E+07	1.352	
			*tet*O	8.00E+08[bd]	5.00E+07	1.204	
			*tet*Q	1.30E+09[bd]	1.00E+08	1.114	
			*tet*W	9.00E+07[bd]	8.00E+06	1.051	
			*tet*X	1.50E+09[bd]	8.00E+08	0.273	
Grit removal + Oxidation ditch + Biological aerated filter	Full scale WWTP serving a population of 94,500. Processes include: Grit Removal, Oxidation Ditch and Biological Aerated Filter. (Data estimated from Figures 2 and 4.)	Hangzhou, China	*int*I1	8.00E+07[bd]	–	2.00	Chen and Zhang, 2013
			*sul*1	8.00E+07[bd]	–	2.63	
			*sul*2	2.00E+08[bd]	–	1.70	
			*tet*M	1.00E+07[bd]	–	3.50	
			*tet*O	4.00E+06[bd]	–	3.75	
			*tet*Q	1.00E+07[bd]	–	3.75	
			*tet*W	1.00E+08[bd]	–	3.35	
Waste stabilization pond	Arctic waste stabilization ponds (WSPs); serving 7542 residents; receiving domestic and hospital waste (WWTP uses a Salsnes filter, effluent is then continuously decanted into Frobisher's Bay. Grab samples taken twice September, 2015 and once November 2015. In September the WWTP was not operational so a waste stabilization pond was used; concentration estimates from figure 3a)	Iqaluit; Baffin Island in the Qikiqtani Region of Nunavut, Canada	16S rRNA	6.00E+06[bd]	3.50E+06	0.234	Neudorf et al., 2017
			*bla*CTX-M	8.00E+00[bd]	1.00E+01	-0.097	
			*bla*TEM	1.50E+01[bd]	6.00E+00	0.398	
			*erm*B	3.70E+01[bd]	1.80E+01	0.313	
			*int*l1	8.60E+01[bd]	4.20E+01	0.311	
			*mec*A	1.00E+01[bd]	1.00E+01	0.000	
			*qnr*S	8.00E+00[bd]	1.30E+01	-0.211	
			*sul*1	9.00E+01[bd]	3.70E+01	0.386	
			*sul*2	9.50E+01[bd]	4.00E+01	0.376	
			*tet*O	3.50E+ 01[bd]	2.70E+01	0.113	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Waste stabilization ponds	Arctic waste stabilization ponds (WSPs); serving 1673 residents (A waste stabilization pond is used, it is emptied into the ocean each year in September. Grab samples taken in September 2013 and 2014, concentration estimates from figure 3c)	Pond Inlet; Baffin Island in the Qikiqtani Region of Nunavut, Canada	16S rRNA	6.00E+07[bd]	5.00E+07	0.079	Neudorf et al., 2017
			*bla*CTX-M	2.10E+01[bd]	2.00E+01	0.021	
			*bla*TEM	7.00E+00[bd]	9.00E+00	-0.109	
			*erm*B	5.50E+01[bd]	4.65E+01	0.073	
			*intl*1	2.50E+01[bd]	2.90E+01	-0.064	
			*mec*A	6.00E+00[bd]	6.50E+00	-0.035	
			*qnr*S	6.80E+01[bd]	7.00E+01	-0.013	
			*sul*1	3.90E+01[bd]	3.20E+01	0.086	
			*sul*2	1.80E+01[bd]	1.90E+01	-0.023	
			*tet*O	3.95E+01[bd]	3.80E+01	0.017	
Waste stabilization ponds	Arctic waste stabilization ponds (WSPs); serving 983 residents (Two waste stabilization ponds are used in series. Grab samples were taken June, July, September 2013 and June, September 2014; concentration estimates from figure 3b)	Clyde River; Baffin Island in the Qikiqtani Region of Nunavut, Canada	16S rRNA	5.00E+06[bd]	2.00E+06	0.398	Neudorf et al., 2017
			*bla*CTX-M	1.40E+01[bd]	8.00E+00	0.243	
			*bla*TEM	3.50E+01[bd]	1.40E+01	0.398	
			*erm*B	4.00E+01[bd]	1.60E+01	0.398	
			*intl*1	1.28E+02[bd]	6.00E+01	0.329	
			*mec*A	8.00E+00[bd]	1.00E+01	-0.097	
			*qnr*S	1.10E+01[bd]	1.00E+01	0.041	
			*sul*1	7.70E+01[bd]	5.30E+01	0.162	
			*sul*2	8.90E+01[bd]	8.40E+01	0.025	
			*tet*O	5.00E+01[bd]	3.40E+01	0.167	
Naturally aerated lagoon	Full WWTP receiving domestic (50%) and pretreated industrial sewage (50%), serving 165,184 people (Grab samples taken in triplicate. 16S rRNA concentration values from table 2, ARG gene/16S rRNA copies values from table S2, absolute abundance was then back-calculated. DNA extraction kit used: DneasyBlood & Tissue Kit)	Moknine, Tunisia	16S rRNA	2.31E+08[bd]	3.67E+08	-0.201	Rafraf et al., 2016
			*bla*CTX-M	5.54E+03[bd]	1.60E+04	-0.461	
			*bla*TEM	3.83E+05[bd]	2.26E+05	0.229	
			*erm*B	1.75E+05[bd]	7.16E+05	-0.612	
			*intI*1	1.36E+07[bd]	4.73E+06	0.459	
			*qnr*A	3.34E+04[bd]	5.82E+05	-1.241	
			*qnr*S	ND[bd]	2.42E+04	≥ -4.38*	
			*sul*1	1.75E+07[bd]	2.21E+06	0.899	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Constructed wetland	Full scale WWTP serving a population of 285,000. WWTP processes include Grit Removal, Oxidation Ditch, and Constructed Wetland. (Data estimated from values reported on Figure 2 and 5)	Hangzhou, China	16S rRNA	1.74E+07[bd]	1.18E+06	1.169	Chen and Zhang, 2013
			*int*I1	2.00E+06[bd]	1.00E+05	1.30	
			*sul*1	5.00E+05[bd]	1.00E+04	1.70	
			*sul*2	8.00E+05[bd]	1.00E+05	0.90	
			*tet*M	6.00E+04[bd]	1.00E+03	1.78	
			*tet*O	4.00E+04[bd]	6.00E+02	1.82	
			*tet*Q	1.00E+05[bd]	8.00E+02	2.10	
			*tet*W	1.00E+06[bd]	3.00E+04	1.52	
Integrated surface flow constructed wetland	ICW treating rural domestic sewage from roughly 4000 people. Operated for 10 years. (Data obtained from Table S7)	Nanchang, Jiangxi province, China; Winter	*int*I1	1.82E+06[bd]	6.36E+05	0.457	Fang et al., 2017
Integrated surface flow constructed wetland	ICW treating rural domestic sewage from roughly 4000 people. Operated for 10 years. (Data obtained from Table S7)	Nanchang, Jiangxi province, China; Summer	*int*I1	2.18E+06[bd]	1.14E+06	0.282	
Integrated surface flow constructed wetland	ICW treating rural domestic sewage from roughly 4000 people. Operated for 10 years. (Data obtained from Table S2)	Nanchang, Jiangxi province, China; Winter	Sum of 14 ARGs (*sul*1, *sul*2, *sul*3, *tet*A, *tet*B, *tet*C, *tet*E, *tet*H, *tet*M, *tet*O, *tet*W, *qnr*S, *qnr*B, *qep*A)	8.41E+06[bd]	1.87E+06	0.653	Fang et al., 2017
Integrated surface flow constructed wetland	ICW treating rural domestic sewage from roughly 4000 people. Operated for 10 years. (Data obtained from Table S2)	Nanchang, Jiangxi province, China; Summer	Sum of 14 ARGs (*sul*1, *sul*2, *sul*3, *tet*A, *tet*B, *tet*C, *tet*E, *tet*H, *tet*M, *tet*O, *tet*W, *qnr*S, *qnr*B, *qep*A)	8.76E+06[bd]	3.55E+06	0.392	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Outa	Log Removal	Reference
Oxidation Ditch (Aerobic treatment)	WWTP with 60,000 m^3 capacity and serving about 300,000 residents from urban and residential areas. WWTP configuration consists of grid screen, anaerobic tank, oxidation ditch (aerobic tank) and UV disinfection plus constructed wetland system prior to finally discharging onto a lake. (Triplicate composite samples were collected over 24-h periods with 3-h intervals. Values reported were estimated from Figure 2.)	Linan City, eastern China	HPC (R2A Agar - No antibiotic)	1.25E+06[ce]	2.78E+06	-0.347	Li et al., 2015
			ARB (Sulfamethoxazole) (R2A Agar + 50.4 mg/L sulfamethoxazole)	5.42E+05[ce]	7.51E+05	-0.142	
			ARB (Tetracycline) (R2A Agar + 16 mg/L tetracycline)	8.81E+04[ce]	1.36E+05	-0.189	
			ARB (Tetracycline + Sulfamethoxazole) (R2A Agar + tetracycline + sulfamethoxazole)	3.68E+04[ce]	6.58E+04	-0.252	
Constructed wetland + UV Disinfection	WWTP with 60,000 m^3 capacity and serving about 300,000 residents from urban and residential areas. WWTP configuration consists of grid screen, anaerobic tank, oxidation ditch (aerobic tank) and UV disinfection plus constructed wetland system prior to finally discharging onto a lake. (Triplicate composite samples were collected over 24-h periods with 3-h intervals. Values reported were estimated from Figure 2.)	Linan City, eastern China	HPC (R2A Agar - No antibiotic)	2.78E+06[ce]	1.00E+06	0.444	Li et al., 2015
			ARB (Sulfamethoxazole) (R2A Agar + 50.4 mg/L sulfamethoxazole)	7.51E+05[ce]	1.36E+05	0.742	
			ARB (Tetracycline) (R2A Agar + 16 mg/L tetracycline)	1.36E+05[ce]	1.33E+04	1.01	
			ARB (Tetracycline + Sulfamethoxazole) (R2A Agar + tetracycline + sulfamethoxazole)	6.58E+04[ce]	4.48E+03	1.167	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]culture-based method; [d]gene copies/ mL; [e]cfu/ mL; – not reported; *values calculated using 1 gene copy/mL as the value for ND, NA or < LOQ; ND = not detected

3.1.3 Centralized wastewater treatment plants (WWTP)

While conventional WWTPs do not appear to reduce the (normalized) integron copy number, they do reduce the diversity of gene cassette arrays measured in the raw wastewater (Stalder et al., 2014), the plasmid resistome (Szczepanowski et al., 2009), and ARGs generally by some 33-98% (Tao et al., 2014). These findings imply aerobic treatment may be beneficial with respect to abating ARGs, but not a complete barrier to AMR. To reduce the cost of aeration, a combined anaerobic-aerobic system is also effective in reducing many but not all ARG types (Christgen et al., 2015), as discussed in Section 3.2.1.

3.1.3.1 Removal by primary settling and sedimentation

Table 8. Grit removal, settling, sedimentation

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Grit Removal	Full scale WWTP serving a population of 285,000. WWTP processes include Grit Removal, Oxidation Ditch, and Constructed Wetland. (Data estimated from values reported on Figure 2)	Hangzhou, China	16S rRNA	8.28E+08	7.11E+08	0.066	Chen and Zhang, 2013
			*int*I1	6.00E+07	8.00E+07	-0.12	
			*sul*1	1.00E+07	1.00E+07	0.00	
			*sul*2	2.00E+07	2.00E+07	0.00	
			*tet*M	5.00E+06	4.00E+07	-0.90	
			*tet*O	4.00E+06	3.00E+07	-0.88	
			*tet*Q	9.00E+06	8.00E+07	-0.95	
			*tet*W	7.00E+07	6.00E+07	0.07	
Grit Removal	Full scale WWTP serving a population of 94,500. Processes include: Grit Removal, Oxidation Ditch and Biological Aerated Filter. (Data estimated from values reported on Figure 2)	Hangzhou, China	16S rRNA	1.56E+09	4.28E+09	-0.438	Chen and Zhang, 2013
			*int*I1	4.00E+07	8.00E+07	-0.30	
			*sul*1	2.00E+07	8.00E+07	-0.60	
			*sul*2	2.00E+07	2.00E+08	-1.00	
			*tet*M	8.00E+06	1.00E+07	-0.10	
			*tet*O	3.00E+06	4.00E+06	-0.12	
			*tet*Q	7.00E+06	1.00E+07	-0.15	
			*tet*W	5.00E+07	1.00E+08	-0.30	
Aerated grit removal tank + flow equalization basin	Full-scale activated sludge WWTP (Grab samples collected during four sampling events between July and December 2010. Mean values estimated from Figure 4.)	East Lansing, MI, USA	16S rRNA	1.00E+10	3.98E+09	0.40	Gao et al., 2012
			*sul*I	2.00E+06	6.31E+05	0.50	
			*tet*O	5.01E+06	6.31E+06	-0.10	
			*tet*W	3.98E+06	3.16E+06	0.10	
Aerated Grit Chamber	Urban WWTP, serving 320,000 inhabitants (Composite samples collected from December 2013-June 2014; ARG concentration estimates from figure 4 showing total ARG abundance)	Shanghai, China	16S rRNA	2.00E+05	9.00E+04	0.35	Gao et al., 2015
			*ere*A	2.63E+05	1.00E+05	0.42	
			*ere*B	1.00E+05	1.20E+04	0.92	
			*erm*A	1.86E+01	1.50E+00	1.09	
			*erm*B	1.50E+05	2.00E+04	0.88	
			*erm*C	8.91E+01	2.00E+01	0.65	
			*mef*A/*mef*E	3.00E+05	4.00E+04	0.88	
			*msr*A/*msr*B	9.12E+00	9.00E+00	0.006	
Fine screen + Grit removal + Primary settling	Full scale WWTP with average daily flow of 150,000 m^3/d, using a cyclic activated sludge system. (WWTP sampled once a month from November 2013 to April 2014. Median values estimated from data presented in Figure S3.)	Harbin, China	16S rRNA	5.35E+08	3.26E+08	0.22	Wen et al., 2016
			*bla*CTX-M	2.00E+04	1.00E+04	0.30	
			*int*I1	7.00E+06	3.00E+06	0.37	
			*sul*1	4.00E+05	2.00E+05	0.30	
			*sul*2	1.00E+07	7.00E+06	0.15	
			*tet*A	4.00E+04	1.00E+04	0.60	
			*tet*O	4.00E+04	1.00E+04	0.60	
			*tet*W	2.00E+06	1.00E+06	0.30	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Primary settling	Large full scale WWTP designed for biological nitrogen removal (Median ARG abundance values from samples collected monthly over a year were estimated from Figure 2. Inlet temp 14 ± 3.3 °C. Outlet temp 14 ± 4.2 °C.)	Gothenburg, Sweden	*mec*A	4.00E+01	1.80E+01	0.35	Borjesson et al., 2009
Secondary settling	Large full scale WWTP designed for biological nitrogen removal (Median ARG abundance values from samples collected monthly over a year were estimated from Figure 2. Inlet temp 14 ± 3.3 °C. Outlet temp 14 ± 4.2 °C.)	Gothenburg, Sweden	*mec*A	4.00E+02	3.00E+00	2.12	Börjesson et al., 2009
Primary Settling	Full scale WWTP serving a population of 2,750,000. Processes include: Headworks, Primary Settling, Anaerobic-Anoxic-Oxic biological treatment, Secondary settling, and UV disinfection. (Data estimated from values reported on Figure 2)	Hangzhou, China	16S rRNA	1.15E+09	1.00E+09	0.061	Chen and Zhang, 2013
			*int*I1	9.00E+07	8.00E+07	0.05	
			*sul*1	1.00E+07	1.00E+07	0.00	
			*sul*2	3.00E+07	3.00E+07	0.00	
			*tet*M	7.00E+06	4.00E+06	0.24	
			*tet*O	3.00E+06	2.00E+06	0.18	
			*tet*Q	1.00E+07	1.00E+07	0.00	
			*tet*W	5.00E+07	4.00E+07	0.10	
Sedimentation tank	Full scale, large municipal WWTP receiving domestic sewage and pretreated hospital sewage (Grab samples collected July 2nd, 2015. Mean and standard deviation values of absolute abundance from Table S2.)	Verbania, Italy	*ars*B	8.50E+05	1.74E+05	0.689	Di Cesare et al., 2015
			*bla*CTX-M	2.63E+03	4.79E+02	0.740	
			*bla*TEM	2.11E+04	4.79E+03	0.644	
			*czc*A	6.36E+04	1.16E+04	0.739	
			*erm*B	7.82E+05	8.50E+04	0.964	
			*int*I1	5.10E+05	1.21E+05	0.625	
			*qnr*S	1.24E+06	4.67E+05	0.424	
			*sul*2	3.36E+05	5.69E+04	0.771	
			*tet*A	1.99E+05	5.05E+04	0.596	
Primary settling	Full-scale activated sludge WWTP (Grab samples collected during four sampling events between July and December 2010. Mean values estimated from Figure 4.)	East Lansing, MI, USA	16S rRNA	3.98E+09	6.31E+09	-0.20	Gao et al., 2012
			*sul*I	6.31E+05	1.00E+06	-0.20	
			*tet*O	6.31E+06	1.58E+06	0.60	
			*tet*W	3.16E+06	1.00E+06	0.50	
Secondary settling	Full-scale activated sludge WWTP (Grab samples collected during four sampling events between July and December 2010. Mean values estimated from Figure 4.)	East Lansing, MI, USA	16S rRNA	1.58E+09	2.00E+08	0.90	
			*sul*I	2.00E+05	1.58E+04	1.10	
			*tet*O	3.98E+04	3.16E+04	0.10	
			*tet*W	3.98E+04	1.58E+04	0.40	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Middle Settling Tank	Urban WWTP, serving 320,000 inhabitants (Composite samples collected from Dec 2013-June 2014; ARG concentration estimates from figure 4 showing total ARG abundance)	Shanghai, China	16S rRNA	1.50E+05	1.00E+04	1.176	Gao et al., 2015
			*ere*A	1.70E+05	6.50E+03	1.418	
			*ere*B	9.00E+03	8.00E+00	3.051	
			*erm*A	6.00E-01	3.00E-01	0.301	
			*erm*B	5.50E+03	7.00E+02	0.895	
			*erm*C	8.00E+00	4.00E-01	1.301	
			*mef*A/*mef*E	2.00E+04	1.00E+02	2.301	
			*msr*A/*msr*B	3.00E+00	5.00E-01	0.778	
Secondary Settling tank	Urban WWTP, serving 320,000 inhabitants (Composite samples collected from Dec 2013-June 2014; ARG concentration estimates from figure 4 showing total ARG abundance)	Shanghai, China	16S rRNA	2.50E+03	2.00E+03	0.097	
			*ere*A	2.00E+03	1.41E+03	0.152	
			*ere*B	5.00E+00	4.90E+00	0.009	
			*erm*A	3.10E-01	1.82E-01	0.231	
			*erm*B	1.90E+02	8.00E+01	0.376	
			*erm*C	4.50E-01	3.63E-01	0.093	
			*mef*A/*mef*E	5.00E+01	6.00E+01	-0.079	
			*msr*A/*msr*B	7.00E-01	6.00E-01	0.067	
Primary Clarifier	Full scale WWTP receiving domestic wastewater and industrial effluents, capacity: 400,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	2.80E+10	1.00E+10	0.447	Li et al., 2016
			*sul*1	4.30E+10	2.90E+10	0.171	
			*sul*2	3.50E+09	3.30E+09	0.026	
			*tet*A	5.00E+09	4.20E+09	0.076	
			*tet*B	1.00E+08	8.00E+07	0.097	
			*tet*C	1.80E+10	1.40E+10	0.109	
			*tet*G	2.60E+09	1.90E+09	0.136	
			*tet*L	1.00E+08	2.00E+08	-0.301	
			*tet*M	7.50E+08	6.00E+08	0.097	
			*tet*O	7.00E+08	8.00E+08	-0.058	
			*tet*Q	5.00E+09	4.50E+09	0.046	
			*tet*W	2.50E+10	1.00E+10	0.398	
			*tet*X	1.80E+09	1.70E+09	0.025	
Secondary Clarifier	Full scale WWTP receiving domestic wastewater and industrial effluents, capacity: 400,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	2.20E+09	5.00E+08	0.643	
			*sul*1	2.60E+09	7.00E+08	0.570	
			*sul*2	5.90E+08	1.60E+08	0.567	
			*tet*A	4.00E+08	5.50E+07	0.862	
			*tet*B	1.00E+07	2.10E+06	0.678	
			*tet*C	9.00E+08	1.70E+08	0.724	
			*tet*G	6.50E+08	3.70E+08	0.245	
			*tet*L	1.20E+07	1.90E+06	0.800	
			*tet*M	3.10E+07	4.20E+06	0.868	
			*tet*O	5.50E+07	9.00E+06	0.786	
			*tet*Q	5.00E+08	2.60E+07	1.284	
			*tet*W	1.50E+09	1.30E+08	1.062	
			*tet*X	1.50E+08	2.60E+07	0.761	
Primary clarifier	Full-scale WWTP receiving domestic sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)	Northern China	16S rRNA	4.30E+08	5.10E+08	-0.074	Luo et al., 2013
			*bla*NDM-1	2.90E+04	2.20E+04	0.12	
	Full-scale WWTP receiving domestic and industrial sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)		16S rRNA	5.70E+07	4.30E+09	-1.878	
			*bla*NDM-1	1.50E+03	2.50E+04	-1.222	
Secondary Clarifier	Full-scale WWTP receiving domestic sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)	Northern China	16S rRNA	2.50E+05	2.00E+03	2.097	
			*bla*NDM-1	6.90E+10	8.40E+07	2.915	
	Full-scale WWTP receiving domestic and industrial sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)		16S rRNA	2.50E+11	1.60E+08	3.194	
			*bla*NDM-1	7.50E+05	2.50E+03	2.477	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Primary clarifier	WWTP1 treating approx. 540,000 m^3/day from a population of 2.1 million. Plant employs anaerobic and anoxic lagoon followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L) (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2.)	Northern China	16S rRNA	4.32E+08	5.36E+08	-0.094	
			erm (*erm*B and, *erm*C)	8.96E+05	5.83E+05	0.187	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	5.47E+04	3.76E+04	0.163	
			sul (*sul*1, *sul*2, and *sul*3)	1.82E+07	1.46E+07	0.096	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T, *tet*W, and *tet*S)	6.49E+05	6.49E+05	0.000	
	WWTP2 treating approx. 580,000 m^3/day from a population of 2.2 million. Plant employs anaerobic and anoxic lagoon followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2.)	Northern China	16S rRNA	5.71E+07	4.32E+09	-1.879	
			erm (*erm*B and, *erm*C)	1.22E+06	2.85E+07	-1.369	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	1.52E+05	2.86E+06	-1.276	
			sul (*sul*1, *sul*2, and *sul*3)	8.80E+06	1.49E+08	-1.23	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T, *tet*W, and *tet*S)	1.51E+06	1.86E+07	-1.09	
Secondary clarifier	WWTP1 treating approx. 540,000 m^3/day from a population of 2.1 million. Plant employs anaerobic and anoxic lagoon followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2.)	Northern China	16S rRNA	7.13E+10	8.63E+07	2.917	Mao et al., 2015
			erm (*erm*B and, *erm*C)	1.73E+08	2.85E+03	4.784	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	2.38E+07	2.07E+03	4.061	
			sul (*sul*1, *sul*2, and *sul*3)	3.71E+09	9.45E+05	3.594	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T, *tet*W, and *tet*S)	8.17E+07	6.44E+04	3.104	
	WWTP2 treating approx. 580,000 m^3/day from a population of 2.2 million. Plant employs anaerobic and anoxic lagoon followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2.)	Northern China	16S rRNA	2.51E+11	1.57E+08	3.202	
			erm (*erm*B and, *erm*C)	4.58E+08	6.77E+05	2.831	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	4.99E+07	6.81E+04	2.865	
			sul (*sul*1, *sul*2, and *sul*3)	2.16E+09	3.55E+06	2.784	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T, *tet*W, and *tet*S)	2.83E+08	4.91E+05	2.761	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible;bqPCR;cgene copies/mL

Table 9. Aerobic and anaerobic secondary treatment.

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Anaerobic/Anoxic/Oxic Biological treatment + Secondary Settling	Full scale WWTP serving a population of 2,750,000. Processes include: Headworks, Primary Settling, Anaerobic-Anoxic-Oxic biological treatment, Secondary settling, and UV disinfection. (Data estimated from values reported on Figure 2)	Hangzhou, China	16S rRNA	1.00E+09[bd]	1.78E+07	1.75	Chen and Zhang, 2013
			*int*I1	8.00E+07[bd]	2.00E+06	1.60	
			*sul*1	1.00E+07[bd]	3.00E+05	1.52	
			*sul*2	3.00E+07[bd]	1.00E+06	1.48	
			*tet*M	4.00E+06[bd]	7.00E+04	1.76	
			*tet*O	2.00E+06[bd]	3.00E+04	1.82	
			*tet*Q	1.00E+07[bd]	1.00E+05	2.00	
			*tet*W	4.00E+07[bd]	1.00E+06	1.60	
Primary settling + Anaerobic process	Municipal WWTP using Oxidation ditch as main treatment process. (Data estimated from Figure 2.)	Hefei, China	16S rRNA	3.39E+08[bd]	1.81E+07	1.273	Li et al., 2017
			*int*I1	7.00E+06[bd]	3.00E+05	1.37	
			*sul*1	2.00E+08[bd]	4.00E+06	1.70	
			*sul*2	8.00E+06[bd]	2.00E+05	1.60	
			*tet*O	1.00E+06[bd]	2.00E+04	1.70	
			*tet*Q	6.00E+06[bd]	1.00E+06	0.78	
			*tet*W	1.00E+05[bd]	3.00E+03	1.52	
Biological treatment + secondary settling	Domestic WWTP with average daily flow of 150,000 m^3 serving population of about 370,000 (Data extracted from Table 2)	Hong Kong	*tet*A	6.00E+07[bd]	2.40E+04	3.398	Zhang et al., 2009
			*tet*C	1.35E+08[bd]	2.27E+05	2.774	
Biological treatment + secondary settling	Domestic WWTP with average daily flow rate of 8,478 m^3 serving population of about 19,000 (Data extracted from Table 2)	Hong Kong	*tet*A	1.59E+08[bd]	6.50E+04	3.388	
			*tet*C	1.90E+08[bd]	3.68E+05	2.713	
Activated Sludge	Large full scale WWTP designed for biological nitrogen removal (Median ARG abundance values from samples collected monthly over a year were estimated from Figure 2. Inlet temp 14 ± 3.3 °C. Outlet temp 14 ± 4.2 °C.)	Gothenburg, Sweden	*mec*A	1.80E+01[bd]	4.00E+02	-1.35	Borjesson et al., 2009
Nitrifying trickling filters	Large full scale WWTP designed for biological nitrogen removal (Median ARG abundance values from samples collected monthly over a year were estimated from Figure 2. Inlet temp 14 ± 3.3 °C. Outlet temp 14 ± 4.2 °C.)	Gothenburg, Sweden	*mec*A	2.00E+00[bd]	8.00E-01	0.40	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Aerobic digestion	Bench-scale (10 L) system fed untreated wastewater solids from full-scale municipal WWTP (Values reported are ARG abundance means over reactor 200 d life, and were estimated from Figures 2 and 3. Aerobic digester was operated at semi-continuous flow conditions at room temperature (DO ≥ 2 mg/L))	–	*erm*B	4.00E+09[bd]	7.00E+07	1.76	Burch et al., 2013
			*int*I1	5.00E+08[bd]	2.00E+09	-0.60	
			*sul*1	1.00E+09[bd]	1.00E+08	1.00	
			*tet*A	7.00E+08[bd]	7.00E+07	1.00	
			*tet*W	6.00E+09[bd]	9.00E+07	1.82	
			*tet*X	9.00E+09[bd]	8.00E+09	0.05	
Aerobic tank	Full scale WWTP receiving domestic wastewater (44%) and pre-treated industrial wastewater (56%) (Composite samples collected October 2012 to September 2013 (except for February 2013), median concentrations estimated from figure 3. 16S values given for sludge samples only.)	Wuxi, Jiangsu Province, China	*int*l1	3.50E+05[bd]	1.20E+06	-0.535	Du et al., 2015
			*sul*1	1.50E+06[bd]	3.50E+06	-0.368	
			*tet*G	9.00E+04[bd]	1.70E+05	-0.276	
			*tet*W	4.50E+04[bd]	2.20E+04	0.311	
			*tet*X	1.40E+06[bd]	1.70E+06	-0.084	
Anaerobic tank	Full scale WWTP receiving domestic wastewater (44%) and pre-treated industrial wastewater (56%) (Composite samples collected October 2012 to September 2013 (except for February 2013), median concentrations estimated from figure 3. 16S values given for sludge samples only.)	Wuxi, Jiangsu Province, China	*int*l1	3.50E+06[bd]	8.00E+05	0.641	Du et al., 2015
			*sul*1	7.50E+06[bd]	4.00E+06	0.273	
			*tet*G	6.00E+05[bd]	2.30E+05	0.416	
			*tet*W	1.00E+06[bd]	8.50E+04	1.071	
			*tet*X	4.50E+06[bd]	2.60E+06	0.238	
Anoxic tank	Full scale WWTP receiving domestic wastewater (44%) and pre-treated industrial wastewater (56%) (Composite samples collected October 2012 to September 2013 (except for February 2013), median concentrations estimated from figure 3. 16S values given for sludge samples only.)	Wuxi, Jiangsu Province, China	*int*l1	8.00E+05[bd]	3.50E+05	0.359	Du et al., 2015
			*sul*1	4.00E+06[bd]	1.50E+06	0.426	
			*tet*G	2.30E+05[bd]	9.00E+04	0.407	
			*tet*W	8.50E+04[bd]	4.50E+04	0.276	
			*tet*X	2.60E+06[bd]	1.40E+06	0.269	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Anaerobic Tank	Full-scale WWTP receiving domestic sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)	Northern China	16S rRNA	5.10E+08[bd]	5.60E+10	-2.041	Luo et al., 2013
			*bla*NDM-1	2.20E+04[bd]	2.10E+05	-0.98	
	Full-scale WWTP receiving domestic and industrial sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)		16S rRNA	4.30E+09[bd]	1.40E+11	-1.513	
			*bla*NDM-1	2.50E+04[bd]	4.90E+05	-1.292	
Anoxic Tank	Full-scale WWTP receiving domestic sewage		16S rRNA	5.60E+10[bd]	5.40E+10	0.016	Luo et al., 2013
			*bla*NDM-1	2.10E+05[bd]	2.10E+05	0.000	
	Full-scale WWTP receiving domestic and industrial sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)		16S rRNA	1.40E+11[bd]	1.20E+11	0.067	
			*bla*NDM-1	4.90E+05[bd]	4.40E+05	0.047	
Aerated Tank	Full-scale WWTP receiving domestic sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)		16S rRNA	5.40E+10[bd]	6.90E+10	-0.106	Luo et al., 2013
			*bla*NDM-1	2.10E+05[bd]	2.50E+05	-0.076	
	Full-scale WWTP receiving domestic and industrial sewage (Composite samples collected in December, 2011. Abundance of genes taken from Table S2.)		16S rRNA	1.20E+11[bd]	2.50E+11	-0.319	
			*bla*NDM-1	4.40E+05[bd]	7.50E+05	-0.232	
First stage anoxic/aerobic system	Urban WWTP, serving 320,000 inhabitants (Composite samples collected from Dec 2013-June 2014; ARG concentration estimates from figure 4 showing total ARG abundance)	Shanghai, China	16S rRNA	9.00E+04[bd]	1.50E+05	-0.222	Gao et al., 2015
			*ere*A	1.00E+05[bd]	1.70E+05	-0.230	
			*ere*B	1.20E+04[bd]	9.00E+03	0.125	
			*erm*A	1.50E+00[bd]	6.00E-01	0.398	
			*erm*B	2.00E+04[bd]	5.50E+03	0.561	
			*erm*C	2.00E+01[bd]	8.00E+00	0.398	
			*mef*A/*mef*E	4.00E+04[bd]	2.00E+04	0.301	
			*msr*A/*msr*B	9.00E+00[bd]	3.00E+00	0.477	
Second-stage anoxic/aerobic system	Urban WWTP, serving 320,000 inhabitants (Composite samples collected from Dec 2013-June 2014; ARG concentration estimates from figure 4 showing total ARG abundance)	Shanghai, China	16S rRNA	1.00E+04[bd]	2.50E+03	0.602	
			*ere*A	6.50E+03[bd]	2.00E+03	0.512	
			*ere*B	8.00E+00[bd]	5.00E+00	0.204	
			*erm*A	3.00E-01[bd]	3.10E-01	-0.014	
			*erm*B	7.00E+02[bd]	1.90E+02	0.566	
			*erm*C	4.00E-01[bd]	4.50E-01	-0.051	
			*mef*A/*mef*E	1.00E+02[bd]	5.00E+01	0.301	
			*msr*A/*msr*B	5.00E-01[bd]	7.00E-01	-0.146	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Anaerobic Tank	Full WWTP receiving domestic sewage from urban and residential areas, serving 300,000 people (Composite samples collected March to May, 2013. Sample concentrations estimated with WebPlotDigitizer from figure S3)	Linan City, China	*int*I1	3.41E+10[bd]	1.16E+10	0.468	Li et al., 2015
			*sul*1	2.09E+10[bd]	9.09E+09	0.362	
			*sul*2	4.62E+09[bd]	1.67E+09	0.442	
			*tet*A	1.76E+09[bd]	7.78E+08	0.355	
			*tet*B	6.95E+07[bd]	4.14E+07	0.225	
			*tet*C	2.07E+09[bd]	8.60E+08	0.381	
			*tet*G	2.01E+09[bd]	1.51E+09	0.124	
			*tet*L	7.91E+07[bd]	4.85E+07	0.212	
			*tet*M	6.18E+08[bd]	3.43E+08	0.256	
			*tet*O	8.70E+09[bd]	4.24E+09	0.312	
			*tet*Q	1.28E+10[bd]	1.18E+10	0.035	
			*tet*W	6.53E+09[bd]	5.04E+09	0.112	
			*tet*X	3.05E+09[bd]	8.85E+08	0.537	
Anaerobic tank	Full scale medium sized WWTP capacity 60,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	2.20E+10[bd]	1.50E+10	0.166	Li et al., 2016
			*sul*1	2.20E+10[bd]	2.00E+10	0.041	
			*sul*2	3.30E+07[bd]	5.00E+06	0.820	
			*tet*A	6.00E+09[bd]	5.00E+09	0.079	
			*tet*B	9.00E+07[bd]	8.00E+07	0.051	
			*tet*C	7.50E+10[bd]	7.00E+10	0.030	
			*tet*G	9.00E+09[bd]	5.00E+09	0.255	
			*tet*L	1.00E+08[bd]	8.00E+07	0.097	
			*tet*M	5.00E+08[bd]	4.50E+08	0.046	
			*tet*O	9.00E+08[bd]	8.00E+08	0.051	
			*tet*Q	4.00E+09[bd]	1.30E+09	0.488	
			*tet*W	8.00E+07[bd]	9.00E+07	-0.051	
			*tet*X	4.50E+09[bd]	1.50E+09	0.477	
Biological Reaction Tank (anaerobic, anoxic, denitrification)	Full scale WWTP receiving domestic wastewater and industrial effluents, capacity: 400,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	1.00E+10[bd]	2.20E+09	0.658	Li et al., 2016
			*sul*1	2.90E+10[bd]	2.60E+09	1.047	
			*sul*2	3.30E+09[bd]	5.90E+08	0.748	
			*tet*A	4.20E+09[bd]	4.00E+08	1.021	
			*tet*B	8.00E+07[bd]	1.00E+07	0.903	
			*tet*C	1.40E+10[bd]	9.00E+08	1.192	
			*tet*G	1.90E+09[bd]	6.50E+08	0.466	
			*tet*L	2.00E+08[bd]	1.20E+07	1.222	
			*tet*M	6.00E+08[bd]	3.10E+07	1.287	
			*tet*O	8.00E+08[bd]	5.50E+07	1.163	
			*tet*Q	4.50E+09[bd]	5.00E+08	0.954	
			*tet*W	1.00E+10[bd]	1.50E+09	0.824	
			*tet*X	1.70E+09[bd]	1.50E+08	1.054	
Biological treatment+ secondary settling	Full-scale medium WWTP serving 40,000 inhabitant equivalents (Values estimated from data presented on Figure 3)	Germany (0.5 km up-stream to the Schussen estuary into Lake Constance)	Resistant *E. coli*	5.00E+04[ce]	2.00E+01	3.398	Lueddeke et al., 2015
			Resistant *Enterococci*	2.00E+03[ce]	2.50E+01	1.903	
			Resistant *Staphylococci*	9.00E+01[ce]	2.00E-01	2.653	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Anaerobic biological treatment	WWTP with 60,000 m^3 capacity and serving about 300,000 residents from urban and residential areas. WWTP configuration consists of grid screen, anaerobic tank, oxidation ditch (aerobic tank) and UV disinfection plus constructed wetland systemprior to finally discharging onto a lake. (Triplicate composite samples were collected over 24-h periods with 3-h intervals. Values reported were estimated from Figure 2.)	Linan City, eastern China	HPC (R2A Agar - No antibiotic)	2.78E+06[ce]	1.25E+06	0.347	Li et al., 2015
			ARB (Sulfamethoxazole) (R2A Agar + 50.4 mg/L sulfamethoxazole)	7.51E+05[ce]	5.42E+05	0.142	
			ARB (Tetracycline) (R2A Agar + 16 mg/L tetracycline)	1.36E+05[ce]	8.81E+04	0.189	
			ARB (Tetracycline + Sulfamethoxazole) (R2A Agar + tetracycline + sulfamethoxazole)	6.58E+04[ce]	3.68E+04	0.252	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]culture-based method; [d]gene copies/ mL; [e]cfu/ mL

3.1.3.3 Activated sludge

Table 10. Activated sludge

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Biological treatment (activated sludge)	Full scale, large municipal WWTP receiving domestic sewage and pretreated hospital sewage. (Grab samples collected July 2nd, 2015. Mean and standard deviation values of absolute abundance from Table S2.)	Verbania, Italy	*ars*B	1.74E+05	1.86E+04	0.971	Di Cesare et al., 2015
			*bla*CTX-M	4.79E+02	<LOQ	≤ 2.68*	
			*bla*TEM	4.79E+03	<LOQ	≤ 3.68*	
			*czc*A	1.16E+04	9.04E+02	1.108	
			*erm*B	8.50E+04	3.14E+03	1.432	
			*int*I1	1.21E+05	3.42E+03	1.549	
			*qnr*S	4.67E+05	9.59E+03	1.687	
			*sul*2	5.69E+04	5.44E+03	1.020	
			*tet*A	5.05E+04	1.16E+03	1.639	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Anaerobic-Aerobic-Aerated biological treatment (with partial return activated sludge not accounted for in process influent)	WWTP1 treating approx. 540,000 m^3/day from a population of 2.1 million. Plant employs anaerobic and anoxic lagoon followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2)	Northern China	16S rRNA	5.36E+08	7.13E+10	-2.124	
			erm (*erm*B and, *erm*C)	5.83E+05	1.73E+08	-2.474	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	3.76E+04	2.38E+07	-2.801	
			sul (*sul*1, *sul*2, and *sul*3)	1.46E+07	3.71E+09	-2.405	
			tet (t*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H,*tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T,*tet*W, and *tet*S)	6.49E+05	8.17E+07	-2.10	
Anaerobic-Aerobic-Aerated biological treatment (with partial return activated sludge not accounted for in process influent)	WWTP2 treating approx. 580,000 m^3/day from a population of 2.2 million. Plant employs anaerobic and anoxic lagoon followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2)	Northern China	16S rRNA	4.32E+09	2.51E+11	-1.763	Mao et al., 2015
			erm (*erm*B and, *erm*C)	2.85E+07	4.58E+08	-1.206	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	2.86E+06	4.99E+07	-1.241	
			sul (*sul*1, *sul*2, and *sul*3)	1.49E+08	2.16E+09	-1.16	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L *tet*O, *tet*Q, *tet*X, *tet*T *tet*W, and *tet*S)	1.86E+07	2.83E+08	-1.183	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Activated sludge (with partial secondary sludge recycling)	Full-scale activated sludge WWTP (Grab samples collected during four sampling events between July and December 2010. Process influent concentrations do not account for recycled sludge. Mean values estimated from Figure 4.)	East Lansing, MI, USA	16S rRNA	6.31E+09	1.58E+09	0.60	Gao et al., 2012
			*sul*I	1.00E+06	2.00E+05	0.70	
			*tet*O	1.58E+06	3.98E+04	1.60	
			*tet*W	1.00E+06	3.98E+04	1.40	
Conventional activated sludge	Full WWTP receiving domestic (60%) pretreated industrial (38%); untreated hospital (2%), serving 127,824 people (Grab samples taken in triplicate. 16S rRNA concentration values from table 2, ARG gene/16S rRNA copies values from table S2, absolute abundance was then back-calculated. DNA extraction kit used: DneasyBlood & Tissue Kit)	Frina (Monastir), Tunisia	16S rRNA	5.84E+08	1.59E+08	0.565	Rafraf et al., 2016
			*bla*CTX-M	1.01E+03	2.64E+05	-2.417	
			*bla*TEM	1.43E+05	9.15E+05	-0.806	
			*erm*B	3.86E+06	1.91E+05	1.306	
			*int*I1	1.68E+06	5.39E+06	-0.506	
			*qnr*A	1.61E+05	5.26E+05	-0.514	
			*qnr*S	1.25E+04	2.30E+06	-2.265	
			*sul*1	2.07E+07	5.91E+06	0.544	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Conventional activated sludge	Full WWTP receiving domestic (90%) and pretreated industrial (10%) sewage, serving 87,277 people (Grab samples taken in triplicate. 16S rRNA concentration values from table 2, ARG gene/16S rRNA copies values from table S2, absolute abundance was then back-calculated. DNA extraction kit used: DneasyBlood & Tissue Kit)	Zaouiet Kontech (Jemmal), Tunisia	16S rRNA	4.39E+08	2.24E+08	0.292	
			*bla*CTX-M	2.59E+05	1.51E+05	0.234	
			*bla*TEM	6.64E+05	5.37E+05	0.092	
			*erm*B	2.47E+07	3.89E+05	1.803	
			*int*I1	5.66E+06	3.16E+06	0.253	
			*qnr*A	2.15E+05	1.00E+05	0.332	
			*qnr*S	2.20E+05	1.95E+05	0.052	
			*sul*1	8.76E+06	3.16E+06	0.443	
Conventional activated sludge	Full WWTP receiving domestic sewage, serving 13,488 people (Grab samples taken in triplicate. 16S rRNA concentration values from table 2, ARG gene/16S rRNA copies values from table S2, absolute abundance was then back-calculated. DNA extraction kit used: DneasyBlood & Tissue Kit)	Sahline, Tunisia	16S rRNA	3.36E+08	1.54E+08	0.339	
			*bla*CTX-M	ND	ND	–	
			*bla*TEM	2.93E+05	3.07E+05	-0.020	
			*erm*B	1.25E+06	7.90E+04	1.199	
			*int*I1	1.09E+06	1.44E+06	-0.121	
			*qnr*A	1.85E+06	1.12E+06	0.218	
			*qnr*S	3.21E+03	ND	≤ 3.51*	
			*sul*1	1.72E+06	6.13E+06	-0.552	
Cyclic activated sludge system	Full scale WWTP with average daily flow of 150,000 m^3/d, using a cyclic activated sludge system. (WWTP sampled once a month from November 2013 to April 2014. Median values estimated from data presented in Figure S3.)	Harbin, China	16S rRNA	3.26E+08	1.23E+07	1.42	Wen et al., 2016
			*bla*CTX-M	1.00E+04	7.00E+02	1.15	
			*int*I1	3.00E+06	2.00E+05	1.18	
			*sul*1	2.00E+05	1.00E+04	1.30	
			*sul*2	7.00E+06	3.00E+05	1.37	
			*tet*A	1.00E+04	4.00E+02	1.40	
			*tet*O	1.00E+04	3.00E+02	1.52	
			*tet*W	1.00E+06	1.00E+04	2.00	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Activated Sludge (prior to secondary sedimentation)	WWTP treating domestic wastewater from population of about 100,000. Daily average flow rate: 15,000 m^3. (Values extracted from Table 3)	Nanjing, China; April 2008	*int*I1	2.04E+07	2.49E+09	-2.087	Zhang et al., 2009
			*tet*A	4.96E+07	4.23E+09	-1.931	
			*tet*C	8.06E+07	4.56E+09	-1.753	
Activated Sludge (prior to secondary settling)	Domestic WWTP with average daily flow of 150,000 m^3 serving population of about 370,000 (Data extracted from Table 3)	Hong Kong	*tet*A	6.00E+07	2.60E+07	0.363	
			*tet*C	1.35E+08	6.70E+07	0.304	
Activated Sludge (prior to secondary settling)	Domestic WWTP with average daily flow rate of 8478 m^3 serving population of about 19,000 (Data extracted from Table 3)	Hong Kong	*tet*A	1.59E+08	2.19E+08	-0.139	Zhang et al., 2009
			*tet*C	1.90E+08	8.06E+08	-0.628	
Activated sludge WWTP with UV disinfection	Full WWTP receiving domestic sewage, serving 16,358 people (Grab samples taken in triplicate. 16S rRNA concentration values from table 2, ARG gene/16S rRNA copies values from table S2, absolute abundance was then back-calculated. DNA extraction kit used: DneasyBlood & Tissue Kit)	Beni Hassen, Tunisia	16S rRNA	2.22E+08	1.43E+08	0.191	Rafraf et al., 2016
			*bla*CTX-M	ND	ND		
			*bla*TEM	4.53E+04	3.94E+05	-0.939	
			*erm*B	1.09E+06	1.27E+05	0.934	
			*int*I1	2.73E+06	2.37E+06	0.061	
			*qnr*A	3.44E+05	1.89E+06	-0.740	
			*qnr*S	3.28E+03	ND		
			*sul*1	2.99E+06	2.79E+06	0.030	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a,b,c]	Concentration Out[a,b,c]	Log Removal	Reference
Sedimentation tank and biological (activated sludge) treatment and chemical (aluminum polychloride enriched by sodium hydroxide) treatment	Full scale, small municipal WWTP receiving domestic sewage (Grab samples collected July 13th, 2015. Mean and standard deviation values of absolute abundance from Table S2.)	Cannobio, Italy	*ars*B	2.63E+05	3.82E+04	0.838	Di Cesare et al., 2015
			*bla*CTX-M	1.91E+03	N/A	≤ 3.28*	
			*bla*TEM	1.63E+04	N/A	≤ 4.21*	
			*czc*A	1.59E+04	7.25E+02	1.341	
			*erm*B	6.71E+05	1.21E+03	2.744	
			*int*I1	2.93E+05	6.43E+03	1.659	
			*qnr*S	7.93E+05	5.10E+02	3.192	
			*sul*2	1.78E+05	2.49E+03	1.854	
			*tet*A	1.39E+05	7.06E+02	2.294	
Sedimentation tank and combined biological (activated sludge) and chemical (aluminum polychloride) treatment	Full scale, large municipal WWTP receiving domestic sewage and pretreated hospital sewage (Grab samples collected July 8th, 2015. Mean and standard deviation values of absolute abundance from Table S2.)	Novara, Italy	*ars*B	1.10E+06	7.98E+04	1.139	Di Cesare et al., 2015
			*bla*CTX-M	4.55E+04	<LOQ	≤ 4.55*	
			*bla*TEM	6.66E+04	<LOQ	≤ 4.82*	
			*czc*A	8.39E+04	3.76E+03	1.349	
			*erm*B	4.62E+06	4.05E+03	3.057	
			*int*I1	9.79E+05	1.45E+03	2.829	
			*qnr*S	5.98E+06	1.14E+04	2.720	
			*sul*2	7.10E+05	1.68E+04	1.626	
			*tet*A	5.57E+05	2.28E+03	2.388	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]culture-based method; [d]gene copies/ mL; [e]cfu/ mL; – not reported; *values calculated using 1 gene copy/mL as the value for ND, NA or < LOQ; ND = not detected

Table 11. Full treatment

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Full WWTP	Full scale activated sludge WWTP A (ARG abundance means estimated from Figure 1.)	Wisconsin, USA; March	*tet*G	1.50E+07[bd]	1.50E+05	2.00	Auerbach et al., 2007
		Wisconsin, USA; July	*tet*G	2.00E+06[bd]	1.50E+04	2.12	
		Wisconsin, USA; November	*tet*G	7.00E+07[bd]	8.00E+05	1.94	
		Wisconsin, USA; March	*tet*Q	9.00E+08[bd]	6.00E+05	3.18	
		Wisconsin, USA; July	*tet*Q	1.50E+07[bd]	8.00E+03	3.27	
		Wisconsin, USA; November	*tet*Q	6.00E+08[bd]	2.00E+05	3.48	
Full WWTP	Full scale activated sludge WWTP B (ARG abundance means estimated from Figure 1.)	Wisconsin, USA; March	*tet*G	1.00E+07[bd]	3.00E+05	1.52	
			*tet*Q	2.50E+08[bd]	1.50E+06	2.22	
Full WWTP	Large full scale WWTP designed for biological nitrogen removal (Median ARG abundance values from samples collected monthly over a year were estimated from Figure 2. Inlet temp 14 ± 3.3 °C. Outlet temp 14 ± 4.2 °C.)	Gothenburg, Sweden	*mec*A	4.00E+01[bd]	2.00E+00	1.30	Borjesson et al., 2009
Full WWTP	Full scale serving 214,000 people, receiving domestic waste and wastewater from health care centers (Samples collected between February, 2010. Samples not extracted with Kit (GOS buffer followed by freezing in liquid nitrogen, thawing, centrifugation etc.). Values estimated from Figure 3.)	Lausanne, Switzerland	16S rRNA	6.56E+07[bd]	3.81E+07	0.236	Czekalski et al., 2012
			*sul*1	1.93E+06[bd]	2.91E+06	-0.178	
			*sul*2	3.71E+05[bd]	3.24E+05	0.059	
Full WWTP (anaerobic/anoxic/aerobic-membrane bioreactor)	Full scale, 56% industrial and 44% domestic waste (Composite samples collected from Nov 6th - 15th, 2012. 16S not reported. Values from Table 4.)	Wuxi, China	*int*l1	1.32E+07[bd]	9.33E+04	2.151	Du et al., 2014
			*sul*1	1.10E+07[bd]	9.33E+04	2.072	
			*tet*G	3.89E+05[bd]	2.45E+03	2.201	
			*tet*W	5.62E+05[bd]	7.08E+02	2.900	
			*tet*X	1.95E+06[bd]	3.89E+04	1.700	
Full WWTP with anaerobic/aerobic treatment	Full scale, Domestic wastewater (Composite samples collected from Nov 6th - 15th, 2012. 16S not reported. Values from Table 4.)	Nanjing, China	*int*l1	7.41E+06[bd]	8.71E+04	1.930	
			*sul*1	7.76E+06[bd]	1.00E+05	1.890	
			*tet*G	4.37E+05[bd]	5.13E+03	1.930	
			*tet*W	3.09E+05[bd]	1.02E+03	2.481	
			*tet*X	1.38E+06[bd]	5.62E+04	1.390	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Full-WWTP (anaerobic/anoxic/aerobic-membrane bioreactor)	Full scale WWTP receiving domestic wastewater (44%) and pre-treated industrial wastewater (56%) (Composite samples collected October 2012 to September 2013 (except for February 2013); average values reported from the range reported in table 4)	Wuxi, Jiangsu Province, China	*int*I1	7.48E+06[bd]	1.82E+05	1.614	Du et al., 2015
			*sul*1	5.28E+07[bd]	4.27E+05	2.092	
			*tet*G	4.67E+05[bd]	8.51E+04	0.739	
			*tet*W	8.69E+05[bd]	1.84E+03	2.674	
			*tet*X	2.84E+06[bd]	2.52E+04	2.052	
Full WWTP	Secondary mechanical-biological treatment plant serving ~120,000 population. No disinfection. The WWTP collects about 55,000 m^3 of wastewater from households (70%), industry (10%), and other sources. (Samples collected four times, seasonally, on December 2013, and February, May and July 2014.)	Poland	16S rRNA	4.60E+08[bd]	4.50E+05	3.010	Makowska et al., 2016
			*int*I1	2.30E+04[bd]	2.10E+03	1.040	
			*sul*1	7.60E+04[bd]	1.50E+04	0.705	
			*sul*2	2.60E+04[bd]	6.20E+03	0.623	
			*tet*A	1.60E+03[bd]	3.50E+02	0.660	
			*tet*B	1.90E+03[bd]	1.80E+01	2.023	
			*tet*M	1.00E+03[bd]	2.50E+01	1.602	
Full WWTP	WWTP treating domestic wastewater from population of about 100,000. Daily average flow rate: 15,000 m^3. (Values extracted from Table 3)	Nanjing, China; April 2008	*int*I1	2.04E+07[bd]	1.20E+06	1.230	Zhang et al., 2009
			*tet*A	4.96E+07[bd]	1.41E+06	1.546	
			*tet*C	8.06E+07[bd]	1.37E+06	1.770	
Full WWTP	Full scale treating municipal waste, average processing capacity of 60,000 m^3/day; includes mechanical, biological, and chemical treatment (Samples collected January, April, July, and October 2015. Values from Table 1.)	Olsztyn, Poland	amoxicillin resistant *Escherichia coli*.	6.40E+04[ce]	9.10E+02	1.847	
Full WWTP	Full scale treating municipal waste, average processing capacity of 60,000 m^3/day; includes mechanical, biological, and chemical treatment (Samples collected January, April, July, and October 2015. Values from Table 1.)	Olsztyn, Poland	tetracycline resistant *Escherichia coli*.	4.21E+04[ce]	1.20E+02	2.545	Osinska et al., 2017
Full WWTP	Full scale treating municipal waste, average processing capacity of 60,000 m^3/day; includes mechanical, biological, and chemical treatment (Samples collected January, April, July, and October 2015. Values from Table 1.)	Olsztyn, Poland	ciprofloxacin resistant *Escherichia coli*.	3.10E+03[ce]	7.50E+01	1.616	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Full WWTP	Secondary mechanical-biological treatment plant serving ~120,000 population. No disinfection. The WWTP collects about 55,000 m^3 of wastewater from households (70%), industry (10%), and other sources. (Samples collected four times, seasonally, on December 2013, and February, May and July 2014)	Poland	Tetracycline resistant (February)	1.90E+04[ce]	2.30E+02	1.917	Makowska et al., 2016
			sulfamethoxazole resistant (February)	1.70E+05[ce]	2.90E+03	1.768	
			Tetracycline resistant (July)	3.10E+04[ce]	3.30E+02	1.973	
			sulfamethoxazole resistant (July)	4.00E+04[ce]	1.30E+03	1.488	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]culture-based method; [d]gene copies/ mL; [e]cfu/ mL

3.1.3.4 Advanced treatment

Table 12. Miscellaneous filters

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Membrane bioreactor	Full scale WWTP receiving domestic wastewater (44%)and pre-treated industrial wastewater (56%) (Composite samples collected October 2012 to September 2013, (except for February 2013) median concentrations estimated from figure 3. 16S values given for sludge samples only).	Wuxi, Jiangsu Province, China	*int*I1	1.20E+06[b,d]	1.90E+04	1.800	2015 Du et al.,
			*sul*1	3.50E+06[b,d]	5.50E+04	1.804	
			*tet*G	1.70E+05[b,d]	3.40E+03	1.699	
			*tet*W	2.20E+04[b,d]	2.00E+02	2.041	
			*tet*X	1.70E+06[b,d]	6.50E+03	2.418	
Peat Biofilter	Household onsite treatment and reuse system serving a 3 bedroom family farm; expected daily volume 1360 L (Samples collected August 2013 to April 2014)	Ohio London,	*tet*Q	1.90E+05[b,d]	1.10E+03	2.237	Park et al., 2016
Chemical treatment (aluminum polychloride enriched by calcium hydroxide and anionic polyacrylamide)	Full scale, large municipal WWTP receiving domestic sewage and pretreated hospital sewage (Grab samples collected July 2nd, 2015. Mean and standard deviation values of absolute abundance from Table S2. ND = not detected.)	Verbania, Italy	*ars*B	1.86E+04[b,d]	1.90E+04	-0.009	Di Cesare et al., 2015
			*bla*CTX-M	<LOQ[b,d]	<LOQ	–	
			*bla*TEM	<LOQ[b,d]	<LOQ	–	
			*czc*A	9.04E+02[b,d]	6.81E+02	0.123	
			*erm*B	3.14E+03[b,d]	4.34E+02	0.859	
			*int*I1	3.42E+03[b,d]	2.70E+03	0.103	
			*qnr*S	9.59E+03[b,d]	1.18E+03	0.910	
			*sul*2	5.44E+03[b,d]	3.11E+03	0.243	
			*tet*A	1.16E+03[b,d]	1.30E+03	-0.049	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Air drying beds	Outdoor 0.6 x 0.6 x 0.6 m3 air drying beds lined with gravel and sand. A mixture of primary and secondary solids were applied and monitored for 100 days (Values reported were maximum ARG abundance from three replicates and estimated from Figure 2. In= untreated solids, Out = solids after 100 days of drying bed operation.)	Southern Minnesota	*erm*B	8.00E+11[b,e]	1.00E+07	4.90	Burch et al., 2013
			*int*I1	4.00E+10[b,e]	2.00E+10	0.30	
			*sul*1	2.00E+11[b,e]	1.00E+11	0.30	
			*tet*A	3.00E+09[b,e]	4.00E+07	1.88	
			*tet*W	1.00E+11[b,e]	2.00E+07	3.70	
			*tet*X	2.00E+09[b,e]	4.00E+07	1.70	
Flocculation-filtration	Full scale medium WWTP serving 40,000 inhabitant equivalents (Values estimated from data presented on Figure 3)	Germany (0.5 km up- stream to the Schussen estuary into Lake Constance)	Resistant *E. coli*	2.00E+01[c,f]	1.00E+01	0.301	
			Resistant *Enterococci*	2.50E+01[c,f]	5.00E+00	0.699	
			Resistant Staphylococci	2.00E-01[c,f]	2.00E-02	1	
GAC filtration	Pilot scale, receiving secondary effluent from full scale medium size WWTP serving 40,000 inhabitant equivalents (Values estimated from data presented on Figure 3)	Germany (0.5 km up- stream to the Schussen estuary into Lake Constance)	Resistant *E. coli*	2.00E+01[c,f]	4.00E+00	0.699	Lueddeke et al., 2015
			Resistant Enterococci	2.50E+01[c,f]	1.00E+00	1.398	
			Resistant Staphylococci	2.00E-01[c,f]	3.00E-02	0.824	
Biological treatment + rapid sand filtration	Urban WWTP receiving sewage from 1.25 million inhabitant equivalents. Average inflow: (Data extracted 432,000 m^3/day from Table 2. Treatment process description: "Activated sludge, sand filtration.) including pre-denitrification and biological oxidation (8 h hydraulic retention time, 30 day sludge retention time)," followed by rapid sand filtration.)	Milan, Italy	Ampicillin-resistant *E. coli* (8 ug/mL)	5.80E+03[c,f]	4.80E+01	2.082	Zanotto et al., 2016
			Ampicillin-resistant *E.coli* (16 ug/mL)	6.80E+03[c,f]	5.40E+01	2.1	
			Ampicillin-resistant *E.coli* (32 ug/mL)	5.60E+03[c,f]	6.40E+01	1.942	
			Cloramphenicol-resistant *E. coli* (16 ug/mL)	1.00E+03[c,f]	2.00E+01	1.699	
			Cloramphenicol-resistant *E. coli* (32 ug/mL)	8.00E+02[c,f]	5.00E+00	2.204	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]culture dependent; [d]gene copies/ mL; [e]gene copies/ g dry weight; [f]CFU/ mL; <LOD = below limit of quantification; – = not detected

3.1.4 Alkailine- anaerobic treatment systems

High pH treatment is often used to sanitize biosolids, which is also effective in reducing ARGs (Munir et al., 2011). Taking this knowledge has also led to the application of anaerobic fermentation at pH 10 (Huang et al., 2016). Not only were ARGs reduced (compared to a neutral pH control) by some 0.4 to 1.4 $\log_{10}$ units, but the high pH also reduced the microbial community structures of potential ARG hosts and ARG-associated naked DNA and bacteriophages (Huang et al., 2016). In a broad comparison of the effects of various wastewater biosolids stabilization technologies (air drying, aerobic digestion, mesophilic anaerobic digestion, thermophilic anaerobic digestion, pasteurization, and alkaline stabilization) alkaline stabilization was amongst the most effective for accelerating decay of *intI*1, *tet*(X), *tet*(A), *tet*(W), *sul*1, *erm*(B), and *qnr*A following soil amendment (Burch et al., 2017). So, providing another example of how changing the microbiome may also assist in reducing AMR risk, which could easily be applied by, for example, a lime-treatment stage for excreta-related solids prior to use.

3.1.5 Sludge/manure management on soils

Sanitation residuals applied to land, even following appropriate sludge/manure treatment to reduce AMR issues (see 3.2.1), could result in re-amplification of ARGs. Therefore, it is of interest to understand how to manage soil amendments containing sludge/manure to encourage further biodegradation. For example, when biosolids were added to sandy and silty-loam soils, from a group of five ARGs and intergrase of class 1 integron (*intI*1), the half-life decay rates were considerable slower than reported for wastewater treatment unit operations such as anaerobic digestion; ranging from 13 days (for *erm*(B), with 100 g of biosolids/manure per kg soil) to 81 days (*intI*1 at 40g.kg^{-1}) (Burch et al., 2014; Fahrenfeld et al., 2014; Sharma et al., 2016).

Table 13. Biosolids treatment Lab storage experiments from dewatered Class B Mesophilic digested sludge.

Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Christiansburg, VA; Storage at 4 °C; 1 month storage	*int*I1	3.00E+08[b,c]	8.00E+08	-0.43	
Christiansburg, VA; Storage at 4 °C; 2 month storage	*int*I1	3.00E+08[b,c]	7.00E+08	-0.37	Miller et al., 2014
Christiansburg, VA; Storage at 4 °C; 4 month storage	*int*I1	3.00E+08[b,c]	4.00E+08	-0.12	
Christiansburg, VA; Storage at 10 °C; 1 month storage	*int*I1	3.00E+08[b,c]	1.00E+09	-0.52	
Christiansburg, VA; Storage at 10 °C; 2 month storage	*int*I1	3.00E+08[b,c]	7.00E+08	-0.37	Miller et al., 2014
Christiansburg, VA; Storage at 10 °C; 4 month storage	*int*I1	3.00E+08[b,c]	1.00E+09	-0.52	
Christiansburg, VA; Storage at 20 °C; 1 month storage	*int*I1	3.00E+08[b,c]	5.00E+08	-0.22	
Christiansburg, VA; Storage at 20 °C; 2 month storage	*int*I1	3.00E+08[b,c]	4.00E+08	-0.12	Miller et al., 2014
Christiansburg, VA; Storage at 20 °C; 4 month storage	*int*I1	3.00E+08[b,c]	5.00E+08	-0.22	

Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Christiansburg, VA; Storage at 4 °C; 1 month storage	*sul*1	2.00E+10[b,c]	1.00E+12	-1.70	
Christiansburg, VA; Storage at 4 °C; 2 month storage	*sul*1	2.00E+10[b,c]	4.00E+12	-2.30	Miller et al., 2014
Christiansburg, VA; Storage at 4 °C; 4 month storage	*sul*1	2.00E+10[b,c]	1.00E+10	0.30	
Christiansburg, VA; Storage at 10 °C; 1 month storage	*sul*1	2.00E+10[b,c]	2.00E+10	0.00	
Christiansburg, VA; Storage at 10 °C; 2 month storage	*sul*1	2.00E+10[b,c]	4.00E+10	-0.30	Miller et al., 2014
Christiansburg, VA; Storage at 10 °C; 4 month storage	*sul*1	2.00E+10[b,c]	2.00E+10	0.00	
Christiansburg, VA; Storage at 20 °C; 1 month storage	*sul*1	2.00E+10[b,c]	7.00E+10	-0.54	
Christiansburg, VA; Storage at 20 °C; 2 month storage	*sul*1	2.00E+10[b,c]	3.00E+10	-0.18	Miller et al., 2014
Christiansburg, VA; Storage at 20 °C; 4 month storage	*sul*1	2.00E+10[b,c]	1.00E+10	0.30	

Data estimated from Figure 1;[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]gene copies/g total solid

3.2 Disinfection

3.2.1 UV, ozone or chlorination disinfection of effluents

Processes that stress, but do not kill, targeted bacteria provide a mechanism to select for stress-resistant biotypes, including those with enhanced uptake of ARGs. For example, using an *E. coli* model, Guo et al. (2015) demonstrated that moderate to high doses of UV (>10 $mJ.cm^{-2}$) or chlorine (>80 mg Cl $min.L^{-1}$) greatly suppressed ARG transfer, but lower levels of chlorination (up to 40 mg $min.L^{-1}$) led to a 2-5 fold increase in conjugative ARG transfer. Similar increased risk of ARG transfer by chlorination has also been reported by others (Rizzo et al., 2013a). The other common oxidant, ozone, also appears less effective, providing 4-log pB10 plasmid removal efficiency at 127.15 mg.min L^{-1}, which was 1.04- and 1.25-fold higher than those required for ARB (122.73 mg.min L^{-1}) and a model non-antibiotic resistant bacterial strain, *E. coli* K-12, (101.4 mg.min L^{-1}), respectively (Pak et al., 2016).

However, when using molecular-methods to collectively assay an array of genes comprising the “resistome,” UV treatment has been reported to only reduce tetX and 16S rRNA genes by 0.58 and 0.60 Log_{10} units, respectively, with other genes reduced 0.36-0.40 even when the dose was increased to 250 $mJ.cm^{-2}$ (Zhang et al., 2015b). Hence, Zhang et al. (2015) recommended a sequential UV/chlorination treatment, to enhance ARG removal, which has also been shown to be effective with 0.05-2.0 $mg.L^{-1}$ chlorination (Lin et al., 2016a).

Another biocide used in sanitation is ammonia nitrogen

(NH_3-N) (Fidjeland et al., 2015), which can also be used in combination with chlorination to enhance ARG removal (1.2-1.5 log_{10} reduction at a Cl_2:NH_3-N ratio over 7.6:1) (Zhang et al., 2015b).

Hence, with due consideration of modes of activity, both UV and chlorination can be effective in reducing ARGs and mobile genetic elements rather than co-selecting for them (Lin et al., 2016b). Overall, known benefits of such disinfection processes for pathogen reduction likely outweigh lesser established concerns regarding potential to enhance AMR.

An important consideration when using molecular methods to assess the effectiveness of disinfectants is that it is essential to employ as long a qPCR amplicon product as possible (e.g., 1,000 bp) in order to capture sufficient DNA damage and for the kinetics to be meaningful (McKinney and Pruden, 2012). Further, a re-growth step following disinfection and before molecular analysis can aid in determining what the net effect of disinfection will be downstream, in terms of selection of potentially more resistant strains.

Table 14. Disinfection

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Chlorine disinfection	WWTP1 treating approx. 540,000 m^3/day from a population of 2.1 million. Plant employs anaerobic and anoxic lagoons followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2.)	Northern China	16S rRNA	8.63E+07[b,d]	2.64E+07	0.513	Mao et al., 2015
			erm (*erm*B and, *erm*C)	2.85E+03[b,d]	1.86E+03	0.187	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	2.07E+03[b,d]	9.73E+02	0.327	
			sul (*sul*1, *sul*2, and *sul*3)	9.45E+05[b,d]	4.45E+05	0.327	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T, *tet*W, and *tet*S)	6.44E+04[b,d]	3.03E+04	0.327	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Chlorine disinfection	WWTP2 treating approx. 580,000 m^3/day from a population of 2.2 million. Plant employs anaerobic and anoxic lagoons followed by a conventional activated sludge process with chlorine disinfection (contact time of 30 min at 5 mg/L (One-liter composite samples collected every 2 h for 24 h from outlet of each treatment unit using a GRASP refrigerated automatic sampler. Data estimated from Figure 2)	Northern China	16S rRNA	1.57E+08[b,d]	1.86E+07	0.928	Mao et al., 2015
			erm (*erm*B and, *erm*C)	6.77E+05[b,d]	1.44E+05	0.673	
			qnr (*qnr*B, *qnr*D, and *qnr*S)	6.81E+04[b,d]	1.37E+04	0.696	
			sul (*sul*1, *sul*2, and *sul*3)	3.55E+06[b,d]	9.84E+05	0.557	
			tet (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*M, *tet*L, *tet*O, *tet*Q, *tet*X, *tet*T, *tet*W, and *tet*S)	4.91E+05[b,d]	1.44E+05	0.534	
Chlorination disinfection	Domestic WWTP with average daily flow rate of 8,478 m^3 serving population of about 19,000 (Data extracted from Table 3)	Hong Kong	*tet*A	6.50E+04[b,d]	2.12E+04	0.487	Zhang et al., 2009
			*tet*C	3.68E+05[b,d]	1.33E+04	1.442	
Chlorine Disinfection	Full scale, large municipal WWTP receiving domestic sewage and pretreated hospital sewage (Grab samples collected July 2nd, 2015. Mean and standard deviation values of absolute abundance from Table S2.)	Verbania, Italy	*ars*B	1.90E+04[b,d]	4.63E+03	0.613	Di Cesare et al., 2015
			*bla*CTX-M	<LOQ[b,d]	<LOQ	–	
			*bla*TEM	<LOQ[b,d]	<LOQ	–	
			*czc*A	6.81E+02[b,d]	1.98E+02	0.536	
			*erm*B	4.34E+02[b,d]	1.90E+02	0.359	
			*int*I1	2.70E+03[b,d]	5.53E+02	0.689	
			*qnr*S	1.18E+03[b,d]	3.99E+02	0.471	
			*sul*2	3.11E+03[b,d]	5.43E+02	0.758	
			*tet*A	1.30E+03[b,d]	3.02E+02	0.634	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Peracetic acid disinfection	Full scale, small municipal WWTP receiving domestic sewage (Grab samples collected July 13th, 2015. Mean and standard deviation values of absolute abundance from Table S2.)	Cannobio, Italy	*ars*B	3.82E+04[b,d]	2.63E+04	0.162	Di Cesare et al., 2015
			*bla*CTX-M	N/A[b,d]	N/A	–	
			*bla*TEM	N/A[b,d]	<LOQ	–	
			*czc*A	7.25E+02[b,d]	5.29E+02	0.137	
			*erm*B	1.21E+03[b,d]	1.84E+03	-0.182	
			*int*I1	6.43E+03[b,d]	7.78E+03	-0.083	
			*qnr*S	5.10E+02[b,d]	1.44E+03	-0.451	
			*sul*2	2.49E+03[b,d]	2.96E+03	-0.075	
			*tet*A	7.06E+02[b,d]	5.33E+02	0.122	
NaClO disinfection + Rapid gravity sand filtration +dichlorination	Full scale activated sludge WWTP (Grab samples collected during four sampling events between July and December 2010. Mean values estimated from Figure 4.)	East Lansing, MI, USA	16S rRNA	2.00E+08[b,d]	6.31E+07	0.500	Gao et al., 2012
			*sul*I	1.58E+04[b,d]	1.00E+04	0.200	
			*tet*O	3.16E+04[b,d]	7.94E+03	0.600	
			*tet*W	1.58E+04[b,d]	5.01E+03	0.500	
Complete mixing batch chlorinatino	Household onsite treatment and reuse system serving a 3 bedroom family farm; expected daily volume 1,360 L (Samples collected August 2013 to April 2014)	London, Ohio, USA	*tet*Q	1.10E+03[b,d]	9.90E+03	-0.954	Park et al., 2016
Chlorination	Full WWTP (ARG abundance means estimated from Figure 4 a and b)	Jeddah, Saudi Arabia	*tet*O	2.00E+02[b,d]	2.50E+02	-0.097	Al-Jassim et al., 2015
			*tet*Q	8.80E+02[b,d]	1.80E+02	0.689	
			*tet*W	1.10E+03[b,d]	4.50E+02	0.388	
			*tet*Z	4.90E+03[b,d]	5.40E+03	-0.042	
Ozonation	Pilot scale, receiving effluent from full scale WWTP (ARG abundance values were extracted from Table 3 and represent the medians calculated from 48 24-h composite samples over 2 years.)	Germany	*amp*C	7.80E+01[b,d]	2.30E+01	0.530	Alexander et al., 2016
			*bla*VIM	8.70E+01[b,d]	7.10E+01	0.088	
			*erm*B	1.40E+02[b,d]	1.10E+00	2.105	
			*van*A	8.70E+02[b,d]	4.30E+02	0.306	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
Ozonation	Pilot scale, receiving secondary effluent from full scale medium size WWTP serving 40,000 inhabitant equivalents (Values estimated from data presented on Figure 3)	Germany (0.5 km up-stream to the Schussen estuary into Lake Constance)	Resistant *E. coli*	2.00E+01[c,e]	1.00E+00	1.301	Lueddeke et al., 2015
			Resistant Enterococci	2.50E+01[c,e]	1.00E-01	2.398	
			Resistant Staphylococci	2.00E-01[c,e]	1.50E-02	1.125	
UV disinfection and constructed wetland system	Full WWTP receiving domestic sewage from urban and residential areas, serving 300,000 people (Composite samples collected March to May, 2013. Sample concentrations estimated with WebPlotDigitizer from figure S3)	Linan City, China	*int*I1	2.88E+09[b,d]	3.84E+09	-0.125	Li et al., 2015
			*sul*1	4.30E+09[b,d]	2.22E+09	0.287	
			*sul*2	7.66E+08[b,d]	6.93E+08	0.043	
			*tet*A	8.50E+07[b,d]	5.60E+07	0.181	
			*tet*B	4.93E+06[b,d]	3.30E+06	0.174	
			*tet*C	1.79E+08[b,d]	1.77E+08	0.005	
			*tet*G	3.90E+08[b,d]	5.67E+08	-0.163	
			*tet*L	1.49E+07[b,d]	3.81E+06	0.592	
			*tet*M	6.39E+06[b,d]	5.06E+07	-0.899	
			*tet*O	6.45E+08[b,d]	1.74E+08	0.569	
			*tet*Q	1.25E+09[b,d]	3.74E+07	1.524	
			*tet*W	3.43E+08[b,d]	1.67E+08	0.313	
			*tet*X	1.67E+08[b,d]	5.12E+08	-0.487	
UV disinfection	Full scale medium sized WWTP capacity 60,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	8.00E+08[b,d]	6.00E+08	0.125	Li et al., 2016
			*sul*1	1.30E+09[b,d]	1.90E+08	0.835	
			*sul*2	5.50E+05[b,d]	2.00E+05	0.439	
			*tet*A	4.00E+08[b,d]	2.60E+08	0.187	
			*tet*B	8.00E+06[b,d]	5.50E+06	0.163	
			*tet*C	3.00E+09[b,d]	2.30E+09	0.115	
			*tet*G	8.50E+08[b,d]	3.00E+08	0.452	
			*tet*L	9.00E+06[b,d]	5.50E+06	0.214	
			*tet*M	2.00E+07[b,d]	1.20E+07	0.222	
			*tet*O	5.00E+07[b,d]	2.60E+07	0.284	
			*tet*Q	1.00E+08[b,d]	6.00E+07	0.222	
			*tet*W	8.00E+06[b,d]	6.50E+06	0.090	
			*tet*X	8.00E+08[b,d]	1.70E+08	0.673	
UV disinfection	Full scale WWTP receiving domestic wastewater and industrial effluents, capacity: 400,000t (Composite samples collected September and October, 2013; concentration values estimated from Figure 3)	Eastern China	*int*I1	5.00E+08[b,d]	4.00E+08	0.097	
			*sul*1	7.00E+08[b,d]	3.70E+08	0.277	
			*sul*2	1.60E+08[b,d]	1.00E+08	0.204	
			*tet*A	5.50E+07[b,d]	4.00E+07	0.138	
			*tet*B	2.10E+06[b,d]	1.60E+06	0.118	
			*tet*C	1.70E+08[b,d]	8.50E+07	0.301	
			*tet*G	3.70E+08[b,d]	1.80E+08	0.313	
			*tet*L	1.90E+06[b,d]	1.70E+06	0.048	
			*tet*M	4.20E+06[b,d]	2.60E+06	0.208	
			*tet*O	9.00E+06[b,d]	7.50E+06	0.079	
			*tet*Q	2.60E+07[b,d]	1.80E+07	0.160	
			*tet*W	1.30E+08[b,d]	1.00E+08	0.114	
			*tet*X	2.60E+07[b,d]	1.20E+07	0.336	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Concentration In[a]	Concentration Out[a]	Log Removal	Reference
UV disinfection	Full scale WWTP with average daily flow of 150,000 m^3/d, using a cyclic activated sludge system. (WWTP sampled once a month from November 2013 to April 2014. Median values estimated from data presented in Figure S3.)	Harbin, China	16S rRNA	1.23E+07[b,d]	7.66E+06	0.301	Wen et al., 2016
			*bla*CTX-M	7.00E+02[b,d]	3.00E+02	0.37	
			*int*I1	2.00E+05[b,d]	1.00E+05	0.30	
			*sul*1	1.00E+04[b,d]	6.00E+03	0.22	
			*sul*2	3.00E+05[b,d]	2.00E+05	0.18	
			*tet*A	4.00E+02[b,d]	1.00E+02	0.60	
			*tet*O	3.00E+02[b,d]	1.00E+02	0.48	
			*tet*W	1.00E+04[b,d]	7.00E+03	0.15	

[a]Removals calculated directly from values reported in the reference, when available, or extracted from the published figures using WebPlotDigitizer or manually when this was not possible; [b]qPCR; [c]culture-dependent; [d]gene copy/ mL; [e]CFU/ mL; – = not reported; <LOQ = below limit of quantification; N/A = negative

Table 15. Treatment with disinfection

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Log Removal[a]	Reference
Full WWTP with membrane biological reactor and UV disinfection	Full scale; Log removal by disinfection (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Traverse City, Michigan USA	16S rRNA	3.60	Munir et al., 2011
			*sul*1	3.40	
			*tet*O	8.00	
			*tet*W	7.90	
Full WWTP with membrane biological reactor and UV disinfection	Full scale; Log removal by physical and biological treatment (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Traverse City, Michigan USA	16S rRNA	3.40	
			*sul*1	2.50	
			*tet*O	7.00	
			*tet*W	6.00	

WWT Process	System Scale (i.e., full, pilot, bench, etc.)	Location	ARG/ARB or bacterial indicator	Log Removal[a]	Reference
Full WWTP with rotary biological contractors and chlorination disinfection	Full scale; Log removal by disinfection (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Romeo, Michigan USA	16S rRNA	2.48	Munir et al., 2011
			*sul*1	2.70	
			*tet*O	4.00	
			*tet*W	3.80	
Full WWTP with rotary biological contractors and chlorination disinfection	Full scale; Log removal by physical and biological treatment (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Romeo, Michigan USA	16S rRNA	2.34	
			*sul*1	2.60	
			*tet*O	4.00	
			*tet*W	3.80	
Activated sludge WWTP with chlorination disinfection	Full scale; Log removal by disinfection (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	East Lansing, Michigan USA	16S rRNA	3.24	
			*sul*1	3.80	
			*tet*O	4.60	
			*tet*W	4.60	
Activated sludge WWTP with chlorination disinfection	Full scale; Log removal by physical and biological treatment	East Lansing, Michigan USA	16S rRNA	2.59	
			*sul*1	2.70	
			*tet*O	4.50	
			*tet*W	4.60	
Activated sludge WWTP with UV disinfection	Full scale; Log removal by disinfection (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Lansing, Michigan USA	16S rRNA	3.33	
			*sul*1	3.90	
			*tet*O	5.00	
			*tet*W	4.20	
Activated sludge WWTP with UV disinfection	Full scale; Log removal by physical and biological treatment (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Lansing, Michigan USA	16S rRNA	2.64	
			*sul*1	2.90	
			*tet*O	4.10	
			*tet*W	3.20	
Oxidative ditch WWTP with UV disinfection	Full scale; Log removal by disinfection (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Imlay, Michigan USA	16S rRNA	2.97	
			*sul*1	2.70	
			*tet*O	4.60	
			*tet*W	4.10	
Oxidative ditch WWTP with UV disinfection	Full scale; Log removal by physical and biological treatment (Samples (2-3) taken between Dec 2008 to Oct 2009; DNA extracted with MagNA pure Compact DNA extraction machine; concentrations not reported for individual sampling sites; log removal estimated from Figure 3)	Imlay, Michigan USA	16S rRNA	2.97	
			*sul*1	2.50	
			*tet*O	4.40	
			*tet*W	3.70	

[a]qPCR

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