On the Generation, Impact and Removal of Antibiotic Resistance in the Water Environment

Shi Fan*

Viterbi School of Engineering, University of Southern California, Los Angeles, 90033, United States

* Corresponding author. Email: savannaalwaysyes@gmail.com

ABSTRACT. Emerging pollutants that have the potential to significantly impact the environment include antibiotics. Antibiotic resistance, or microorganisms' ability to withstand medications intended to kill them, can have an impact on a variety of facets of daily life. One of the most vital resources for life, water, contains antibiotic resistance. This review explains where antibiotics and antibiotic resistance genes come from, how they contaminate the aquatic environment, and how they get into water bodies. Since wastewater is the most prominent growth environment for the production and amplification of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), this review particularly explains the causes of antibiotics and the production of antibiotic-resistant bacteria and resistance genes in wastewater treatment plants, as well as antibiotic resistance mechanisms and detection techniques. Since detection techniques are necessary to the study of antibiotic resistance, different detection techniques will also be described in this paper. In addition, ARB and their genes are not only present in hospital wastewater, but also persist in surrounding surface water, river sediments, and river wildlife, even after the water has been purified. Therefore, this review will describe a class of widely used biological treatment technologies—anaerobic digestion—to eliminate antibiotics and antibiotic genes. Anaerobic digestion is divided into many classes, the most common of which is the anaerobic membrane bioreactor. Finally, an outlook for future research is presented.

1. INTRODUCTION

1.1 Antibiotics and Antibiotics resistance

Antibiotics are made from waste or synthetic versions of microorganisms, such as actinomycetes, and their main purpose is to stop the growth of other types of microorganisms or to kill them. Most of the time, antibiotics are used against human or animal pathogens, such as pathogenic bacteria. This means that people can use them to treat most diseases caused by microbial infections such as bacteria, rickettsia, and mycoplasma. Antibiotic resistance existed long before humans used antibiotics to treat disease and will continue to emerge and evolve as a result of competition among microorganisms for limited resources and survival conditions [1].

Species of bacteria that are able to withstand at least one antibiotic exhibit antibiotic resistance. In general, pathogens like bacteria and fungi become resistant when they learn how to beat the drugs that are meant to kill them. Multiple drug resistance (MDR), or "superbugs," are pathogens that are considered resistant to multiple antibiotics [2].

1.2 Long-term infection of Antibiotics resistance

Antibiotic resistance is a normal and natural phenomenon. Nevertheless, uncontrolled human use has led to a rapid deterioration of this phenomenon to the point where it is seriously affecting the lives of humans and other species.The emergence of antibiotic resistance has first resulted in an increase in bacteria carrying antibiotic resistance genes (ARGs)[2]. This gene is the one that worries people the most, and it has been labeled as an emerging environmental contaminant. Because antibiotic-resistant bacteria can replicate, they can transfer antibiotic-resistant genes to more bacteria through gene transfer. As a result, genes that are resistant to antibiotics have spread [3]. Recent research has also revealed that microplastics in wastewater make excellent carriers of genes for antibiotic resistance. Microplastics are not just standalone contaminants but can also transport additional damaging adsorbed contaminants because of hydrophobic and electrostatic interactions. Similarly for antibiotic resistant bacteria, microplastics are good carriers for carrying them. Microplastics themselves, due to their large specific surface area,

enable more antibiotic-resistant bacteria to attach and carry out gene transfer and bacterial colonization on their surfaces. The long-term use of non-degradable plastics by humans pollutes the water and gives room for the development of antibiotic resistance [4].

2. ANTIBIOTICS RESISTANCE IN WATER ENVIRONMENT

2.1 How do antibiotics and ARB/ARGs get in to the water environment

Antibiotic resistance can be transmitted between humans, animals and the environment. Once antibiotics enter the ecosystem, they gradually affect the function of ecologic in this system [5-7]. First, animals are treated by consuming antibiotics, and then resistance can develop. From there, the animal carries antibiotic-resistant bacteria in its body [8]. When people fertilize crops with animal manure, these antibiotic-resistant bacteria can contaminate these plants [8]. Eventually these resistant bacteria spread into the water environment due to irrigation and fertilization [8]. In addition, when patients take antibiotic drugs for treatment, they can also develop resistance. The resistant bacteria can spread to more people through poor sanitation or close contact [8]. These antibiotic-resistant bacteria will eventually appear in the water environment through domestic sewage and medical wastewater. Of course, humans and animals can also become carriers of resistance genes through the consumption of contaminated vegetables and proteins [9].

Fernando Baquero et al. summarized the four major genetic reactors of antibiotic resistance [3]. The first level of reactors involves mainly therapeutic or prophylactic antibiotics and consists of human microbiota and animal microbiota. Tier II reactors are places like ranches and medical clinics where there are many individuals who could become ill and where microbes can without much of a stretch spread. Tertiary reactors primarily encompass effluent from secondary reactors as well as wastewater treatment plant effluent and sludge, where a variety of bacteria undergo genetic reactions resulting in the spread of resistance genes. The fourth stage reactor is a soil, groundwater, and surface water environment that can receive bacterial organisms from the first three reactors and offset each other. Accordingly, the second, third, and fourth reactors characterize human and animal microbiota that continuously integrate with bacteria in the environment, a situation that can substantially increase genetic variation while re-emerging new resistance mechanisms [10].

2.2 Antibiotics resistance in wastewater treatment plant

Wastewater treatment plants (WWTPs) include many different types of processes that alter the fate of antibiotics, ARBs, and ARGs in various ways, hence influencing the development and spread of antibiotic resistance in the environment [11]. The distribution of bacterial populations undergoes significant qualitative and quantitative changes during the wastewater treatment process. It is commonly anticipated that therapy will significantly reduce bacterial populations, including the number of antibiotic-resistant bacteria. However, this is not true for every treatment.

The main antibiotic-resistant bacteria in municipal wastewater are Enterobacteriaceae of human and animal origin, and Aeromonas representing aquatic bacteria [12]. Wastewater, even when treated in wastewater treatment plants, can contain more populations of various resistant bacteria than surface water [13,14]. The samples obtained from WWTPs by Sujatha et al. revealed the presence of ARBs [15]. Researchers analyzed samples for fecal coliforms, E. coli, and enterococci from influent, clarifier outflow, and disinfected discharge water. The results of the study showed that the samples were resistant to ciprofloxacin, methicillin and vancomycin [15]. In addition, Auerbach et al. detected and analyzed tetracycline resistance in wastewater treatment plants (WWTP) by qualitative PCR and quantitative PCR (qPCR) and found that all compartments evaluated in WWTP contained a considerable number of tetR gene types, tetR gene concentrations, and community fractions carrying tetR genes, suggesting WWTP as a potential source of resistance potential source of transmission [16].

Another method of antibiotic resistance is horizontal gene transfer (HGT) from donor bacteria [17]. Although resistance already exists, it can also develop spontaneously or as a result of HGT from donor bacteria, phages, or free DNA [18]. HGT is interceded by donor microscopic organisms, phages, free DNA and could actually happen from dead to living cells [17]. WWTPs have now become a hotspot for HGT because of their complex and diverse microbial populations, which can lead to a wider spread of ARGs [19]. That is dense bacterial populations in WWTPs facilitate genetic exchange between populations and communities, as integrons spread before transposons and plasmids.

In the process of horizontal gene transfer, bacteria acting as vectors transmit ARGs to the host microbiome, and because of their ability to promote gene recombination (via transposons or integrons), this inevitably leads to increased harm from ARGs [20].

Anna C. Shore et al. showed through their study that the mobile Staphylococcal cassette chromosome mec (SCC mec) XI is capable of horizontal transfer between different S. aureus clones[21].

Thus even if there was a relatively small number of ARBs that were first released into the environment by wastewater treatment plants, additional bacteria have become antibiotic-resistant or even multi-antibiotic-resistant as a result of gene transfer [22]. Moreover, scientists have shown that non-pathogenic microbial species with resistance genes can serve as ecological reservoirs for pathogenic bacteria [23].

The detection of ARGs from WWTPs can be broadly divided into sample sampling and DNA extraction, qualitative polymerase chain reaction, real-time quantitative analysis using qPCR, and finally, statistical

analysis and drawing conclusions. The specific methods for detecting ARGs in the environment will be described in Part 3.

3. TECHNIQUES FOR THE DETECTION OF ENVIRONMENTAL ARGs

Techniques for detecting ARG in the environment are divided into two main categories, culture-based methods and culture-independent detection techniques. There are many types of culture-independent techniques, such as qPCR, macrogenomics, etc [24,25].

3.1 Culture-based detection

Culture-based tests can evaluate the potential for proliferation or gene transfer of certain ARBs in various growth environments. The main advantage of culture-based techniques is the ability to confirm phenotypic characteristics, which makes it more beneficial for researchers to discover ecological information about the colony. In addition, this technique allows the enumeration of live cells and enables the assessment of multidrug resistance phenotypes without the need for sophisticated equipment and high level of expertise [26].

However, Manaia et al. pointed out that the bias generated during the culture process can lead to the inability to accurately quantify ARBs and that the culture environment itself is not fully representative of the diversity of the entire biome [20]. With the advent of more abundant non-culture detection techniques, time-consuming and costly culture-based methods are gradually being replaced. The main methods currently used to assess wastewater resistome are quantitative PCR (qPCR) as well as macrogenomics[27].

3.2 Quantitative PCR

Genotype identification is faster and more precise than culture-based assays, and it permits the monitoring of gene-based resistance. Quantitative real-time PCR (qRT-PCR) is a technique for resistance-mediated gene quantification that allows real-time tracking of amplification of gene fragments by enabling specific primers and fluorescent dyes and estimating initial concentrations based on changes in amplification concentrations [28].

The most common fluorochromes and probes are SYBR Green (a double-stranded intercalator) and TaqMan (based on double-labeled oligonucleotides and Taq polymerase exonuclease activity). SYBR Green is less sensitive but also less expensive, so it is used more often [15].

The quantitative real-time polymerase chain reaction (qRT-PCR) technique is widely used to examine the impact of various environmental circumstances and multiple treatments on the eradication of specific ARG, in addition to measuring the spread of ARG in the environment. Auerbach et al. utilized qPCR to detect tetracycline resistance in WWTP and discovered that effluent contained the lowest concentrations of tetQ and tetG on average, with low resistance in effluent gene abundance being primarily attributed to suspended solids concentration and UV disinfection having no direct effect on reducing the number of tet genes in wastewater effluent [16].

Because qRT-PCR itself is very specific, it cannot design primers for unknown genes, which means that it is possible that some genetic variants of ARGs cannot be detected.

3.3 Macrogenomics

Macrogenomics can then be used to overcome the limitations of qRT-PCR itself. It is able to sequence the entire macrogenome, which means that specificity bias can be overcomed. Macrogenomics is the genetic examination of environmental samples' genomes. It can be separated into two categories: "full shotgun metagenomics," which is solely utilized for functional and sequence studies of the collective genome; and "sequence-based metagenomics." The second technique is "marker gene amplification metagenomics" (i.e., 16S ribosomal RNA gene), which is used to evaluate certain genes for polymerase chain reaction (PCR) amplification [29]. Full shotgun metagenomics enables complete sequencing of the genomes available in most communities, as well as analysis of the functional gene composition of known and unknown microbial communities [30].

Macrogenomics has been widely used to study the diversity of ARGs and ARBs in wastewater [31], also it is one of the non-targeted research methods for the discovery of new ARGs. It is important to note that as high-throughput sequencing technology develops, the application cost of macrogenomics is gradually dropping, making its widespread usage by researchers more attractive.

However, it cannot make inferences about antibiotic resistance phenotypes and therefore can limit risk assessment [24].

4. ANAEROBIC DIGESTION(AD) OF ANTIBIOTICS AND ARGs REMOVAL

One of the technologies that is commonly utilized for the elimination of ARGs is known as anaerobic treatment technology. Because it does not require a significant supply of oxygen, and because the methane that is created can also be used as a renewable energy resource if it is put to the right use. When compared to the high prices and shortcomings of producing dangerous byproducts of treatment methods such as advanced oxidation process, adsorption, and electrocoagulation, the advantages of AD become rather apparent [32]. Firstly, the energy demand in the AD process is not high and therefore the cost is relatively low. In addition, AD produces a very low amount of sludge while removing a high amount of organic matter. More importantly, AD does not produce a large amount of toxic by-products or

metabolites, which reduces the pressure on the subsequent treatment.

Aziz et al. provided a summary of the primary roles of anaerobic digestion via (1) anaerobic treatment, (2) the inhibitory that antibiotics effect the production of bioenergy, (3) alterations in the microbial makeup and antibiotic exposure; (4) the role of thermophilic and thermophilic AD as well as additives in the elimination of ARGs. For antibiotic and ARG removal, anaerobic treatment techniques include anaerobic sequencing batch reactors (ASBR), up-flow anaerobic sludge bed reactors (UASB), extended granular sludge bed reactors (EGSB), and anaerobic membrane bioreactors (AMBR) [33].

4.1 Anaerobic sequencing batch reactor (ASBR)

The most typical anaerobic reactor is the anaerobic sequencing batch reactor (ASBR). Aydin et al. examined the effect of various antibiotic combinations on the removal of efflux pump genes (tetA, tetB, tetC, tetD, tetE), ribosome protection genes (tetM, tetS, tetQ, tetW), and individual enzymatic modification genes (tetX), three distinct classes of ARGs in ASBR [34]. They found that the effect of the antibiotic combination on ARGs in anaerobic processes was much greater than the sum of their individual effects. In addition, although ASBR was able to achieve a minimum of 80% COD removal efficiency, the removal of tetA, tetB, sul1, sul2 and ermB genes was low [34].

4.2 Upflow anaerobic sludge bed reactor (UASB)

The upflow anaerobic sludge bed reactor (UASB) works very stably under anaerobic conditions. In experiments using UASB to degrade antibiotics, the alkalinity ratio and the concentration of methane produced remained largely within a range, and even the organic removal rate was consistently around 90% [35]. According to this study, the removal efficiencies of CBZ, DZP, DCF, FLX, and IBP were all less than 15%, but UASB showed good removal rates for SMX, TMP, and NPX [35]. In addition, these researchers found that the higher the biodegradation rate of the antibiotics, the more methane was produced [35].

In high salinity pharmaceutical wastewater, Shi et al. identified a single UASB with an organic loading rate of 8.11 0.31 g COD/L/d and a hydraulic retention period of 41.3 2.2% 48 h for COD removal efficiency[36]. In contrast, when UASB was combined with other reactors (i.e., UASB + SBR and UASB + MBR), the COD removal rate improved to more than 90%[36]. It was determined that the organic load could be reduced significantly by using a UASB in conjunction with either an MBR or an SBR, respectively[36]. Using a combination of UASB, anoxic-aerobic cell (A/O), and advanced oxidation technologies (UV, Ozonation, Fenton, and Fenton/UV), Hou et al. accomplished the simultaneous removal of 18 antibiotics and 10 ARGs. In addition, this study revealed that UASB had a crucial role in the removal of antibiotics, but the Fenton/UV process was the most crucial for the removal of ARGs[37]. In addition, this study demonstrated that UASB played a significant role in the removal of antibiotics, but the Fenton/UV process was most important to treat ARGs. In many modern studies, therefore, a hybrid of anaerobic and aerobic treatment methods is used to enhance removal efficiency[37].

4.3 Expanded granular sludge bed reactor (EGSB)

Expanded granular sludge bed reactor (EGSB) is a modified version of UASB. However, when Hou et al. used the EGSB and MBR reactor composition to treat tetracycline wastewater, the EGSB reactor could only remove 24.5% of hygromycin, 27.7% of tetracycline and 15.7% of chrysomycin, despite the high overall removal of COD, TN and TSS [38]. In addition, when Meng et al. studied the effect of cephalosporins (CFX) on ARG removal in an EGSB reactor and found that adding CFX to the wastewater had no influence on the target ARG content in the sludge [39]. Reduce the sludge concentrations of BlaCTX-M, sul2, qnrS, and AmpC genes in the EGSB reactor system handling antibiotic wastewater [16].

4.4 Anaerobic Membrane Bioreactor (AnMBR)

The AnMBR is now the most widely used anaerobic treatment method for the elimination of antibiotics and ARGs. The membranes in it are selective for specific bacteria passing through them. AnMBR technology can drastically decrease antibiotic re sistance gene copies [40]. In AnMBR antibiotics can be removed by biosorption, biodegradation, and membrane adsorption. Harb et al. compared the effects of aerobic MBR and AnMBR on the abundance of ARGs under the same treatment conditions. They found that antibiotic-type organic micropollutants (OMPs) were more readily biodegraded by anaerobic MBR, i.e., the concentrations of sulfonamide and methomyl resistance genes in anaerobic MBR were found to be 1-2 logs lower than their aerobic counterparts during normalization of 16S rRNA gene copies [41]. Also, Harb et al. demonstrated that the average and highest relative frequency values of sul1, sul2, intl1 and dfrA5 genes in anaerobic reactor sludge were more than one magnitude larger than those in aerobic reactors, reinforcing the fact that AnMBR has a clear advantage to remove ARGs [41].

Zarei-Baygi et al. investigated the removal of antibiotics and the reduction of abundance of ARGs by AnMBR when treating wastewater with the same concentrations of the antibiotics erythromycin (ERY), sulfamethoxazole (SMX) and ampicillin (AMP)[41]. The experimental results showed a reduction of 89-98% for AMP, 69-78% for SMX and 40-58% for ERY in the reactor effluent [41]. For ARGs abundance variation, they found increasing abundance of ARGs in activated sludge, while low abundance of target ARGs in AnMBR

effluent contained 53.6% of sul1 and 31% of intI1, and <1% of tetO and ermF [42].

It is worth mentioning that although membrane contamination generally has an adverse effect on experiments, studies in recent years have found that membrane contamination can be used as a treatment technique to remove ARGs. In order to demonstrate the impact that membrane contamination has on the AnMBR's ability to prevent the re-release of ARGs as a result of fouling, Zarei-Baygi et al. used a system that included three membranes with varying degrees of buildup [43]. The findings indicated that there was a general trend toward a lower absolute abundance of ARGs in the biomass that was suspended. When compared to the suspended biomass, the abundance of ARGs was much higher in the fouling layer, which points to the possibility of adsorption of ARGs in biofilms or horizontal gene transfer of ARGs between biofilms [41, 42].

The majority of researchers have begun to use or focus on the combination of AnMBR and other therapeutic technologies to enhance antibiotic clearance and lower ARGs and ARBs at this point in time. For example, anaerobic rotating membrane bioreactors (AnSRMBR), and anaerobic osmotic membrane bioreactors (AnOMBRs) [29]. Combining AnMBR with microbial fuel cells (MFC) and microbial electrolytic cells is the most talked-about innovative combo technology (MEC) [44]. Ren et al. invented a combined treatment process consisting of a biofuel cell and an anaerobic fluidized bed membrane bioreactor (MFC-AFMBR), which was able to achieve 92.5% effluent tCOD removal and almost complete removal of total suspended solids (TSS) in the same hydraulic retention time [44].

5. CONCLUSION

Antibiotic resistance is innate to the environment and is aggravated by the abuse of antibiotics by humans [8]. This is a significant reason why it poses a hazard to the ecosystem. Antibiotic resistance is primarily a result of the proliferation of ARBs and ARGs. Currently, wastewater is the most favorable environment for the multiplication of antibiotic-resistant bacteria; therefore, wastewater treatment plants must understand the mechanism of ARGs transfer and take appropriate measures to eliminate ARBs and ARGs. The AnMBR is the most prevalent technique which is used to remove ARGs and ARBs, among the numerous available. Research on the eradication of antibiotic resistance is no longer restricted to a single treatment method, but instead mixes AnMBR with a number of other treatment technologies. However, detailed research on the interaction between distinct ARGs and their growth on different substrates are still lacking, making it impossible to remove ARGs and ARBs consistently from complicated wastewater environments. Additionally, this will be the focus of future research.

REFERENCES

1. Larsson, D. G. J., & Flach, C.-F. (2021). Antibiotic resistance in the environment. *Nature Reviews Microbiology*, *20*, 1–13. https://doi.org/10.1038/s41579-021-00649-x
2. Fair, R. J., & Tor, Y. (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, *6*(6), PMC.S14459. https://doi.org/10.4137/pmc.s14459
3. Baquero, F., Martínez, J.-L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*, *19*(3), 260–265. https://doi.org/10.1016/j.copbio.2008.05.006
4. Golwala, H., Zhang, X., Iskander, S. M., & Smith, A. L. (2021). Solid waste: An overlooked source of microplastics to the environment. *Science of the Total Environment*, *769*, 144581. https://doi.org/10.1016/j.scitotenv.2020.144581
5. Bouki, C., Venieri, D., & Diamadopoulos, E. (2013). Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. *Ecotoxicology and Environmental Safety*, *91*, 1–9. https://doi.org/10.1016/j.ecoenv.2013.01.016
6. Thiele-Bruhn, S., & Beck, I.-C. (2005). Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere*, *59*(4), 457–465. https://doi.org/10.1016/j.chemosphere.2005.01.023
7. Kotzerke, A., Sharma, S., Schauss, K., Heuer, H., Thiele-Bruhn, S., Smalla, K., Wilke, B.-M., & Schloter, M. (2008). Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure. *Environmental Pollution*, *153*(2), 315–322. https://doi.org/10.1016/j.envpol.2007.08.020
8. *Infographic - How antibiotic resistance can spread | Antimicrobial resistance*. (2017, November 9). Antimicrobial Resistance. https://www.amr.gov.au/resources/infographic-how-antibiotic-resistance-can-spread
9. *Antibiotic resistance: How does antibiotic resistance spread?* (2014, November 18). European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/en/publications-data/antibiotic-resistance-how-does-antibiotic-resistance-spread
10. *Understanding Antibiotic Resistance in Water: A One Health Approach | One Health | CDC*. (2021, December 9). Www.cdc.gov. https://www.cdc.gov/onehealth/in-action/understanding-antibiotic-resistance-in-water.html
11. Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review.

Science of the Total Environment, *447*, 345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032

12. Bhattacherjee, J. W., Pathak, S. P., & Gaur, A. (1988). Antibiotic resistance and metal tolerance of coliform bacteria isolated from Gomati River water at Lucknow city. *The Journal of General and Applied Microbiology*, *34*(5), 391–399. https://doi.org/10.2323/jgam.34.391
13. Huang, J.-J., Hu, H.-Y., Liu, S.-Q., Li, Y., & Tang, F. (2012). Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. *Environment International*, *42*, 31–36. https://doi.org/10.1016/j.envint.2011.03.001
14. Karkman, A., Do, T. T., Walsh, F., & Virta, M. P. J. (2018). Antibiotic-Resistance Genes in Waste Water. *Trends in Microbiology*, *26*(3), 220–228. https://doi.org/10.1016/j.tim.2017.09.005
15. Nagulapally, S. R., Ahmad, A., Henry, A., Marchin, G. L., Zurek, L., & Bhandari, A. (2009). Occurrence of Ciprofloxacin-, Trimethoprim-Sulfamethoxazole-, and Vancomycin-Resistant Bacteria in a Municipal Wastewater Treatment Plant. *Water Environment Research*, *81*(1), 82–90. https://doi.org/10.2175/106143008x304596
16. Auerbach, E. A., Seyfried, E. E., & McMahon, K. D. (2007). Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water Research*, *41*(5), 1143–1151. https://doi.org/10.1016/j.watres.2006.11.045
17. Sharma, N., & Sharma, S. K. (2021). Wastewater Treatment Plants as emerging source of antibiotic resistance. *Green Chemistry and Water Remediation: Research and Applications*, 239–269. https://doi.org/10.1016/b978-0-12-817742-6.00008-6
18. Dodd, M. C. (2012). Potential impacts of disinfection processes on elimination and deactivation of antibiotic resistance genes during water and wastewater treatment. *Journal of Environmental Monitoring*, *14*(7), 1754. https://doi.org/10.1039/c2em00006g
19. Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.-N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology*, *13*(5), 310–317. https://doi.org/10.1038/nrmicro3439
20. Manaia, C. M. (2017). Assessing the Risk of Antibiotic Resistance Transmission from the Environment to Humans: Non-Direct Proportionality between Abundance and Risk. *Trends in Microbiology*, *25*(3), 173–181. https://doi.org/10.1016/j.tim.2016.11.014
21. Shore, A. C., Deasy, E. C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R., & Coleman, D. C. (2011). Detection of Staphylococcal Cassette ChromosomemecType XI Carrying Highly DivergentmecA,mecI,mecR1,blaZ, andccrGenes in Human Clinical Isolates of Clonal Complex 130 Methicillin-ResistantStaphylococcus aureus. *Antimicrobial Agents and Chemotherapy*, *55*(8), 3765–3773. https://doi.org/10.1128/aac.00187-11
22. Tucker, K., Stone, W., Botes, M., Feil, E. J., & Wolfaardt, G. M. (2022). Wastewater Treatment Works: A Last Line of Defense for Preventing Antibiotic Resistance Entry Into the Environment. *Frontiers in Water*, *4*. https://doi.org/10.3389/frwa.2022.883282
23. Salyers, A., & Shoemaker, N. B. (2006). Reservoirs of Antibiotic Resistance Genes. Animal Biotechnology, 17(2), 137–146. https://doi.org/10.1080/10495390600957076
24. Manaia, C. M., Rocha, J., Scaccia, N., Marano, R., Radu, E., Biancullo, F., Cerqueira, F., Fortunato, G., Iakovides, I. C., Zammit, I., Kampouris, I., Vaz-Moreira, I., & Nunes, O. C. (2018). Antibiotic resistance in wastewater treatment plants: Tackling the black box. *Environment International*, *115*, 312–324. https://doi.org/10.1016/j.envint.2018.03.044
25. Zhang, X.-X., Zhang, T., & Fang, H. H. P. (2009). Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology*, *82*(3), 397–414. https://doi.org/10.1007/s00253-008-1829-z
26. Berney, M., Hammes, F., Bosshard, F., Weilenmann, H.-U., & Egli, T. (2007). Assessment and Interpretation of Bacterial Viability by Using the LIVE/DEAD BacLight Kit in Combination with Flow Cytometry. *Applied and Environmental Microbiology*, *73*(10), 3283–3290. https://doi.org/10.1128/aem.02750-06
27. Botes, M., de Kwaadsteniet, M., & Cloete, T. E. (2012). Application of quantitative PCR for the detection of microorganisms in water. Analytical and Bioanalytical Chemistry, 405(1), 91–108. https://doi.org/10.1007/s00216-012-6399-3
28. Dang, H., Zhang, X., Song, L., Chang, Y., & Yang, G. (2007). Molecular determination of oxytetracycline-resistant bacteria and their resistance genes from mariculture environments of China. Journal of Applied Microbiology, 103(6), 2580–2592. https://doi.org/10.1111/j.1365-2672.2007.03494.x
29. Maaz, M., Yasin, M., Aslam, M., Kumar, G., Atabani, A. E., Idrees, M., Anjum, F., Jamil, F., Ahmad, R., Khan, A. L., Lesage, G., Heran, M., & Kim, J. (2019). Anaerobic membrane bioreactors for wastewater treatment: Novel configurations, fouling control and energy considerations. *Bioresource Technology*, *283*, 358–372. https://doi.org/10.1016/j.biortech.2019.03.061
30. Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G. A., Papanikolaou, N., Kotoulas, G., Arvanitidis, C., & Iliopoulos, I. (2015). Metagenomics: Tools and Insights for Analyzing

Next-Generation Sequencing Data Derived from Biodiversity Studies. *Bioinformatics and Biology Insights*, *9*, 75–88. https://doi.org/10.4137/BBI.S12462

31. Tang, J., Bu, Y., Zhang, X.-X., Huang, K., He, X., Ye, L., Shan, Z., & Ren, H. (2016). Metagenomic analysis of bacterial community composition and antibiotic resistance genes in a wastewater treatment plant and its receiving surface water. *Ecotoxicology and Environmental Safety*, *132*, 260–269. https://doi.org/10.1016/j.ecoenv.2016.06.016
32. Li, Z., & Yang, P. (2018). Review on Physicochemical, Chemical, and Biological Processes for Pharmaceutical Wastewater. *IOP Conference Series: Earth and Environmental Science*, *113*, 012185. https://doi.org/10.1088/1755-1315/113/1/012185
33. Aziz, A., Sengar, A., Basheer, F., Farooqi, I. H., & Isa, M. H. (2021). Anaerobic digestion in the elimination of antibiotics and antibiotic-resistant genes from the environment – A comprehensive review. *Journal of Environmental Chemical Engineering*, 106423. https://doi.org/10.1016/j.jece.2021.106423
34. Aydin, S., Ince, B., & Ince, O. (2015). Development of antibiotic resistance genes in microbial communities during long-term operation of anaerobic reactors in the treatment of pharmaceutical wastewater. *Water Research*, *83*, 337–344. https://doi.org/10.1016/j.watres.2015.07.007
35. Alvarino, T., Suarez, S., Lema, J. M., & Omil, F. (2014). Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. *Journal of Hazardous Materials*, *278*, 506–513. https://doi.org/10.1016/j.jhazmat.2014.06.031
36. Shi, X., Lefebvre, O., Ng, K. K., & Ng, H. Y. (2014). Sequential anaerobic–aerobic treatment of pharmaceutical wastewater with high salinity. *Bioresource Technology*, *153*, 79–86. https://doi.org/10.1016/j.biortech.2013.11.045
37. Hou, J., Chen, Z., Gao, J., Xie, Y., Li, L., Qin, S., Wang, Q., Mao, D., & Luo, Y. (2019). Simultaneous removal of antibiotics and antibiotic resistance genes from pharmaceutical wastewater using the combinations of up-flow anaerobic sludge bed, anoxic-oxic tank, and advanced oxidation technologies. *Water Research*, *159*, 511–520. https://doi.org/10.1016/j.watres.2019.05.034
38. Hou, J., Wang, C., Mao, D., & Luo, Y. (2015). The occurrence and fate of tetracyclines in two pharmaceutical wastewater treatment plants of Northern China. *Environmental Science and Pollution Research*, *23*(2), 1722–1731. https://doi.org/10.1007/s11356-015-5431-5
39. Meng, L., Wang, J., Li, X., & cui, F. (2020). Insight into effect of high-level cephalexin on fate and driver mechanism of antibiotics resistance genes in antibiotic wastewater treatment system. *Ecotoxicology and Environmental Safety*, *201*, 110739. https://doi.org/10.1016/j.ecoenv.2020.110739
40. D. Kappell, A., K. Kimbell, L., D. Seib, M., E. Carey, D., J. Choi, M., Kalayil, T., Fujimoto, M., H. Zitomer, D., & J. McNamara, P. (2018). Removal of antibiotic resistance genes in an anaerobic membrane bioreactor treating primary clarifier effluent at 20 °C. *Environmental Science: Water Research & Technology*, *4*(11), 1783–1793. https://doi.org/10.1039/C8EW00270C
41. Zarei-Baygi, A., Wang, P., Harb, M., Stadler, L. B., & Smith, A. L. (2020). Membrane Fouling Inversely Impacts Intracellular and Extracellular Antibiotic Resistance Gene Abundances in the Effluent of an Anaerobic Membrane Bioreactor. *Environmental Science & Technology*, *54*(19), 12742–12751. https://doi.org/10.1021/acs.est.0c04787
42. Zarei-Baygi, A., Harb, M., Wang, P., Stadler, L. B., & Smith, A. L. (2020). Microbial community and antibiotic resistance profiles of biomass and effluent are distinctly affected by antibiotic addition to an anaerobic membrane bioreactor. *Environmental Science: Water Research & Technology*, *6*(3), 724–736. https://doi.org/10.1039/c9ew00913b
43. Zarei-Baygi, A., Harb, M., Wang, P., Stadler, L. B., & Smith, A. L. (2019). Evaluating Antibiotic Resistance Gene Correlations with Antibiotic Exposure Conditions in Anaerobic Membrane Bioreactors. *Environmental Science & Technology*, *53*(7), 3599–3609. https://doi.org/10.1021/acs.est.9b00798
44. Ren, L., Ahn, Y., & Logan, B. E. (2014). A Two-Stage Microbial Fuel Cell and Anaerobic Fluidized Bed Membrane Bioreactor (MFC-AFMBR) System for Effective Domestic Wastewater Treatment. *Environmental Science & Technology*, *48*(7), 4199–4206. https://doi.org/10.1021/es500737m