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Review

A Mini-Review of Antibiotic Resistance Drivers in Urban Wastewater Treatment Plants: Environmental Concentrations, Mechanism and Perspectives

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Abstract: Antibiotic resistance is one of the biggest challenges to public health and ecological safety in the 21st century. Urban wastewater treatment plants (UWTPs), as reservoirs of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB), simultaneously contain a wide variety of chemical pollutants. The review introduces the actual concentration levels and the mechanisms of antibiotic resistance drivers (ARDs) in UWTPs, including antibiotics, heavy metals, disinfectants, cosmetics and personal care products, non-antibiotic drugs, and microplastics. Moreover, this review emphasizes the importance of approaching the actual activated sludge environment in future research and proposes future directions.

Keywords: antibiotic resistance; urban wastewater treatment plants; co-selection; horizontal gene transfer; emerging pollutants

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1. Introduction

Antibiotic resistance is one urgent and huge challenge to current and future human societies [1]. Urban wastewater treatment plants (UWTPs) are crucial "sink–source" reservoirs for the transmission of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) in environments [2–4]. As a crucial "sink", sewage treatment systems stockpile ARGs and ARB-enriched urban wastewater from multiple sources, for instance, inhabitants [5–8], hospitals [9–11], industries [12,13], antibiotic factories [14–16], farms and slaughterhouses [17–19], etc. Meanwhile, UWTPs discharge ARGs and ARB into various environmental mediums, including receiving water [20], surrounding air [21], and residual sludge treatment [22], posing a higher risk to city residents due to the potential horizontal gene transfer (HGT) of ARGs. Therefore, the development of antibiotic resistance in UWTPs continuously attracts attention.

Recently, antibiotic resistance monitoring reports revealed that the occurrence, abundance, diversity, and mobilization of ARGs in UWTPs are increasing over time [23,24], inviting the exploration of the drivers of antibiotic resistance for researchers. Many studies have consistently pointed out that it could be caused by the overuse and misuse of antibiotics in the human medical field and the livestock and poultry farming industry [25,26]. This has illustrated the causes of increasing antibiotic resistance in the influent stage of UWTPs. However, what happens during the stay of ARGs and ARB in UWTPs cannot be neglected. With intensive biomass, abundant microbial diversity, and well-mixed characteristics, the activated sludge is regarded as a perfect environment for bacteria engaging in HGT to exchange ARGs for each species in UWTPs, confirming previous studies [27,28]. Notably, whether or not bacterial species that received ARGs gain a competitive advantage

in the activated sludge environment in UWTPs is particularly critical, determining the survival and the increasing abundance of these species that benefit from HGT of ARGs. Hence, a systematic review of selection pressure and its determining chemicals in UWTPs is imminent.

Firstly, the inputting of residual antibiotics from urban domestic residential, hospital, medical and suburban animal farming industries could exert selection pressure for bacterial communities in activated sludge in UWTPs [29]. Recently, increasing studies have found that some non-antibiotic materials have the driving ability to promote bacterial antibiotic resistance, including heavy metals [30,31], pesticides [32], disinfectants [33], non-antibiotic drugs [34,35], endocrine disruptors, persistent organic pollutants [36], nanomaterials, microplastics [37], etc. Unfortunately, these antibiotic resistance drivers (ARDs) are continuously inputted into the UWTPs with multiple sources and multiple pathways, and the types and concentrations of potential ARDs in urban wastewater are increasing with the development of human society. In activated sludge, these ARDs not only independently exert selection pressure to promote antibiotic resistance, but also synergistically act on bacterial communities to promote HGT and to select resistant bacteria, which makes activated sludge a significant emerging place of the intensive training and free exchange required for bacterial antibiotic resistance. Therefore, this article aimed at providing insight into the mechanisms of bacterial antibiotic resistance promoted by potential ARDs in UWTPs.

2. Antibiotics

Antibiotics naturally have evolved and been used to treat bacterial infections in humans and animals for a long period, to inhibit microbes and repress bacterial colonization and infection within the body at therapeutic concentrations [38]. Under long-term selection pressure, bacteria could acquire antibiotic resistance through mutations and horizontal gene transfer [39]. The main mechanisms of antibiotic resistance in bacteria include the following: (i) preventing the contact of antibiotics and targets by reducing membrane permeability and increasing efflux pump systems; (ii) altering the antibiotic targets via mutations in the antibiotic target-encoding gene; (iii) target modification through methylation, peptide repeat protein binding, lipopolysaccharide change, and remodeling of the membrane's phospholipid content; and (iv) antibiotic modification with hydrolysis inactivation and chemical group transfer inactivation [38]. Urban wastewater contains various residual antibiotics, which may originate from domestic sewage [40], medical wastewater [41], livestock and poultry farming wastewater [42], and partly from antibiotic production wastewater [43]. However, antibiotics are generally found in urban wastewater at low (sub-inhibitory) concentrations [26,44–50], and the concentrations of beta-lactam antibiotics in urban wastewater have been detected at 1.66–1.38 $\times$ 10^4 μg/L [44,51], 6.4 $\times$ 10^4 μg/L cephalosporins [44], 6 $\times$ 10^3–7 $\times$ 10^3 μg/L streptomycin [45], 0.46–5.6 $\times$ 10^3 μg/L sulfonamides [26,46,52], 0.74–7.9 $\times$ 10^3 μg/L quinolones [47,48,53,54], 3.16 $\times$ 10^{-3}–2.2 $\times$ 10^3 μg/L tetracyclines [49,55], 0.13–1.0 $\times$ 10^4 μg/L macrolides [49,56–58], 1.4 $\times$ 10^3–2.9 $\times$ 10^3 μg/L ciprofloxacin [49], 6.5 $\times$ 10^2–7.3 $\times$ 10^2 μg/L norfloxacin [49], 4.2 $\times$ 10^2–6.5 $\times$ 10^2 μg/L ofloxacin [49] and 0.06–7.9 $\times$ 10^3 μg/L metronidazole [26,50]. Figure 1 shows the summarized concentration ranges of various antibiotics in UWTPs influents and effluents, reported in previous studies (details shown in Table S1). In recent years, increasing studies have confirmed that subtherapeutic doses of antibiotics could promote the emergence of bacterial resistance [25,59–63]. Tian et al. [62] found that 100 μg/L streptomycin and tetracycline significantly increased the abundance of ARGs in actual urban wastewater. When the concentrations reached 5 $\times$ 10^3 μg/L and 2.5 $\times$ 10^4 μg/L, respectively, the proportions of ARGs showed a significant increase. Gullberg et al. [61] tested the effects of low dosages of tetracycline, ciprofloxacin, and streptomycin on ARB by transducing antibiotic-resistant strains of *Escherichia coli* and *Salmonella enterica*. The results show that the lowest promoting antibiotic resistance concentrations were 15 μg/L (~1/100 of the minimum inhibitory concentration, MIC) for tetracycline, 0.1–2.5 μg/L (~1/230–1/10 MIC) for ciprofloxacin,

and 1×10^3 μg/L (~1/4 MIC) for streptomycin. Furthermore, the study also demonstrated that a concentration of 8×10^3 μg/L of streptomycin was able to promote *rpsL105* gene mutations of *Salmonella typhimurium*, which encode ribosomal protein S12 to generate resistance to streptomycin. It should be noted that the above concentrations of tetracycline, ciprofloxacin, and streptomycin were 146 times, 560–290,000 times and 6–7 times higher than that of the promoting threshold of ARB, respectively. Bengtsson-Palme et al. (2016) predicted the no-effect concentrations for resistance selection on 111 antibiotics ranging from 8×10^{-3} to 64 μg/L [29], indicating that the concentrations of antibiotics in UWTPs had a higher no-effect threshold compared with the detected concentrations in previous studies (Figure 1). In conclusion, the residual antibiotics in urban wastewater, which exceed the thresholds that affect bacteria, play a positive role in the abundance of ARGs and ARB in wastewater and subsequent treatment processes, and can enrich resistant mutant strains and change the growth rates of different species to promote antibiotic resistance in microbial communities.

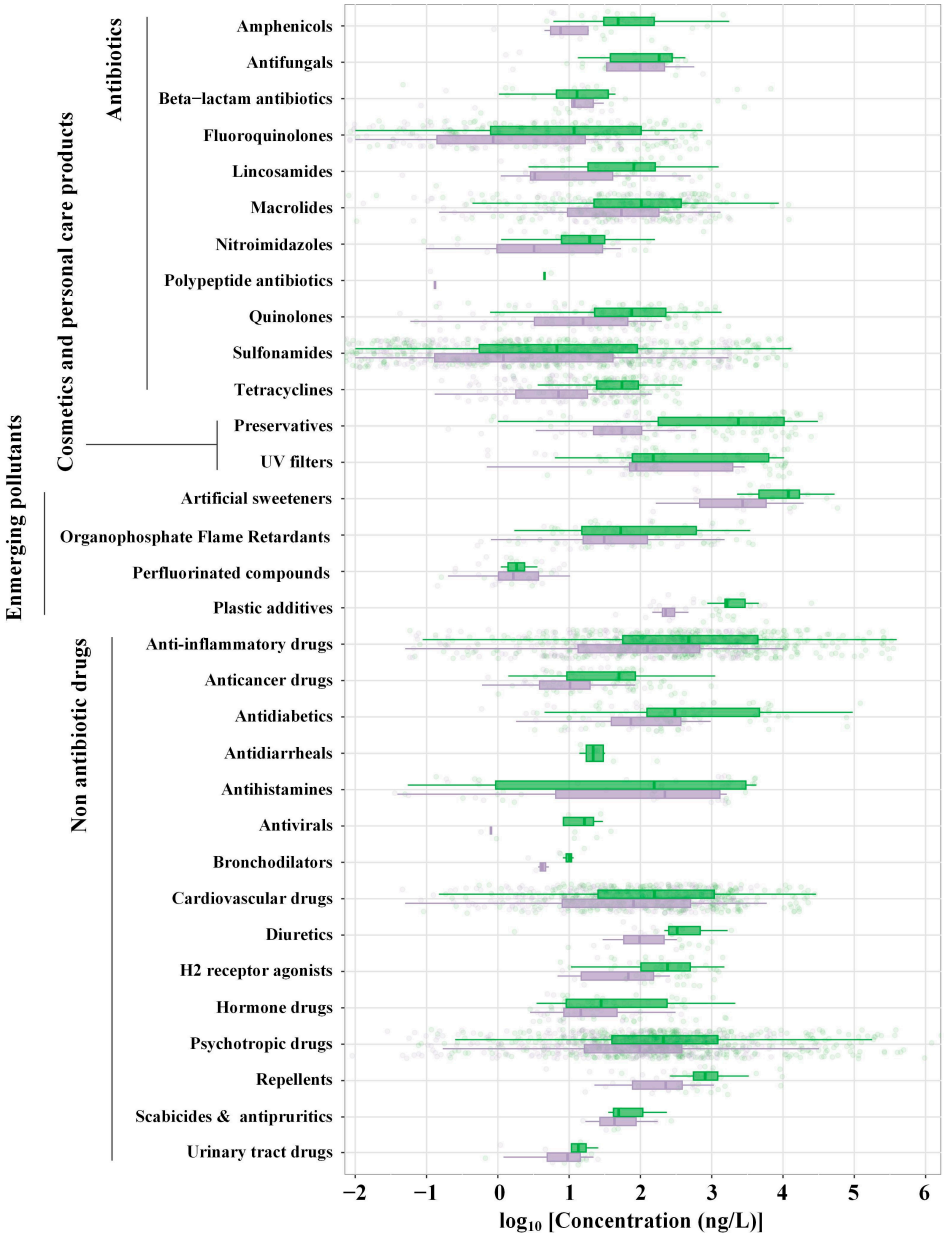


Figure 1. The summarized concentration ranges of antibiotic resistance drivers in urban wastewater treatment plants (UWTPs), that is, UWTPs influent (green) and UWTPs effluent (purple).

3. Heavy Metals

Industrialization and urbanization have led to the release and accumulation of metal ions in urban wastewater. Studies have shown that the concentrations of copper in urban wastewater range from 19.8 to 541 μg/L, while those of nickel range from 2.4 to 17 μg/L, cobalt from 0.41 to 4.60 μg/L, zinc from 38 to 670 μg/L, cadmium from 18 to 24 μg/L, lead from 1.07 to 10 μg/L, silver from 0.05 to 6.5 μg/L, gold from 0.20 to 0.86 μg/L, and mercury from 0.08 to 11 μg/L [64,65], etc. Moreover, the concentrations of heavy metals in urban sewage sludge reached ~mg/kg levels in a national survey [66], including copper at levels from 60.11 to 2436.00 mg/kg, nickel at 0.20 to 692.94 mg/kg, chromium at 2.34 to 4288.00 mg/kg, lead at 1.66 to 294.60 mg/kg, zinc at 50.00 to 13,933.00 mg/kg, cadmium at 0.08 to 210.33 mg/kg, mercury at 0.05 to 28.83 mg/kg, arsenic at 2.00 to 289.13 mg/kg, and manganese at 72.36 to 1259.00 mg/kg, and these also indirectly showed the high concentrations of heavy metals in urban wastewater. Previous studies have revealed co-resistance between metals and antibiotics [31,67,68]. Baker-Austin et al. [30] summarized the co-selectivity between metals and antibiotics, classifying it into three mechanisms: co-resistance, cross-resistance, and co-regulation. In addition, metals serve various functions, specifically including the promotion of reactive oxygen species formation within bacterial cells, the induction of the SOS response, the enhancement of cell membrane permeability, the facilitation of the horizontal gene transfer of antibiotic resistance genes, and the increased risk of ARG transfer to pathogenic bacteria, ultimately leading to the emergence of multidrug-resistant pathogenic bacteria (superbugs) [69].

Extensive research has demonstrated numerous multi-resistance efflux pumps' concurrent extrusion of antibiotics and metals [70,71]. The tetracycline efflux pump gene *tetL* has been found to extrude cobalt, indicating cross-resistance with tetracycline [72]. The oxidoreductase disulfide bond protein (DsbA-DsbB) in *Burkholderia cepacia* has been confirmed by susceptibility tests to exhibit cross-resistance to cadmium, zinc and antibiotics, including β-lactams, kanamycin, erythromycin, novobiocin and ofloxacin [71]. The major facilitator superfamily efflux pump (MdrL) in *Listeria monocytogenes* has been shown to simultaneously pump out cobalt, cadmium, zinc, clindamycin and erythromycin [73]. The two-component regulatory system (BaeSR) in *Salmonella typhimurium* promoted cross-resistance between copper, zinc and antibiotics, including oxacillin, β-lactams, novobiocin and deoxycholate, by increasing the expression of the resistance–nodulation–division-type efflux pump (AcrD) and multidrug transporter ABC (MdtABC) [74]. In a comprehensive bacterial genomic analysis conducted by Li et al. [75], the co-resistance between metals and antibiotics revealed the presence of numerous metal resistance genes (23 metal resistance gene types) and antibiotic resistance genes (including β-lactams, macrolide, lincosamide, streptogramin, bacitracin, aminoglycoside, tetracycline, kasugamycin, polymyxin, sulfonamide, chloramphenicol, fosfomycin, fosmidomycin, quinolone, rifamycin, trimethoprim and vancomycin) on transposons, integrative elements, and plasmids, which included aminoglycoside ARGs–nickel resistance genes, macrolide–iron, macrolide–arsenic, macrolide–aluminum, macrolide–copper, macrolide–zinc, beta-lactam–mercury, beta-lactam–arsenic and beta-lactam–zinc, etc. Perron et al. [76] discovered that *Pseudomonas aeruginosa* could resist both metals (including cobalt, zinc and cadmium) and antibiotics (including imipenem and ciprofloxacin) through the co-regulation of the cobalt–zinc–cadmium resistance protein (CzcR-CzcS).

4. Disinfectants, Cosmetics and Personal Care Products

Disinfectants, such as alcohols, aldehydes, halogens, biguanides, phenols, quaternary ammonium compounds and peroxides [77], are widely used for the disinfection of medical settings, public spaces, households and livestock farms [78]. Studies have shown various residual disinfectants in urban wastewater, which include 0.5–1.3 μg/L N, N-diethyl-3-methylbenzamide [79,80], 1.7–12.7 μg/L triclosan [80–82], chlorhexidine, 0.06 μg/L methylparaben [79], 2.6 μg/L atrazine [80], 99 μg/L quaternary ammonium salts [64], 2.4 μg/L hexachlorophene [64], 2–80 μg/L benzalkonium chloride [83] and 27–105 μg/L chloroxylenol [81]. During the COVID-19 pandemic, disinfectants were used

more than in the pre-pandemic, so that more residual disinfectants were input into urban sewage [84]. Moreover, the overuse and misuse of disinfectants was eased during the period of coexistence with the COVID-19 virus, but remained high compared to the pre-pandemic era [84,85]. During the COVID-19 pandemic, for example, the concentration of residual chlorine reached 0.22 mg/L in urban wastewater, while residual chlorine was not detected in the pre-pandemic era [84]. In recent years, the issue of bacterial antibiotic resistance arising from disinfectants has garnered significant attention [86–89]. Particularly during the COVID-19 pandemic, the widespread use of disinfectants has raised concern about the exacerbation of bacterial antibiotic resistance [90,91].

Cosmetics and personal care products also contain various preservatives and UV filters. Triclosan was widely used in preservatives to add to various products, such as hand soap, toothpaste, deodorant, surgical scrubs, shower gel, hand lotion, hand cream, and mouthwash [92]. Figure 1 shows that the concentration range of preservatives was 0.001–50 μg/L in UWTPs influents, reported in previous studies (details shown in Table S1). Recent studies have shown that triclosan could promote the horizontal transfer of ARGs [87] through mutations and the overexpression of target genes, such as *fab*I, *inh*A, and *mab*A [93,94]. Previous studies have confirmed that triclosan has cross-resistance and co-regulation with antibiotics [95], in which these co-selection mechanisms were usually undertaken through efflux pump genes, such as efflux–pump systems in *Escherichia coli*, including RND-based tripartite efflux pumps comprising the outer membrane protein, the periplasmic membrane fusion protein and the inner membrane transporter (AcrAB-TolC) [96], *Pseudomonas aeruginosa* including multidrug resistance efflux pumps (MexAB-OprM, MexCD-OprJ, and MexEF-OprN) [97], and mutations in regulators promoting efflux pumps, such as *nfx*B [97] and *mex*L [95]. Complex microbial community exposure experiments revealed that triclosan facilitated the horizontal gene transfer of plasmid-carried ARGs into opportunistic pathogens in activated sludge environments, and confirmed that this mechanism was related to excessive oxidative stress, increasing membrane permeability, and SOS response [98]. Recently, benzalkonium chloride as a new antimicrobial agent to replace triclosan and triclocarban has been widely used in consumer products and hospitals [99]. A microcosm exposure experiment of the benzalkonium chloride disinfectant of urban sewage showed that the absolute abundance of ARGs in a 1 mg/L dose exposure was 3.63 times compared to controls [100]. Another microcosm experiment confirmed that benzalkonium chloride had changed the genotype and phenotype of antibiotic resistance [101]. Xing et al. [32] demonstrated that 2.5 mg/L of insecticide could promote resistance in *Escherichia coli* to erythromycin and also induce cross-resistance with ampicillin to promote resistance to ciprofloxacin, chloramphenicol and tetracycline. Buffet-Bataillon et al. [102] elaborated on the mechanism of quaternary ammonium compounds promoting bacterial resistance to antibiotics, for instance, inhibiting efflux pump expression, facilitating horizontal gene transfer and inducing co-resistance and cross-resistance. The mechanism of chlorination disinfection promoting bacterial resistance to antibiotics facilitated the horizontal gene transfer of ARGs, co-resistance and cross-resistance [103]. In addition, compounds like protamine, aminoglycosides, quinolones, acriflavine, benzalkonium chloride, diquat dibromide and pentachlorophenol showed the promotion of bacterial resistance to antibiotics by upregulating the expression of efflux pump systems [104].

5. Non-Antibiotic Drugs

In the current century, bacteria are evolving resistance to antibiotics much faster than new antibiotic drugs are being developed, putting humans at a disadvantage in this race [105]. Therefore, non-antibiotic drugs are recruited to combine with antibiotics or directly treat bacterial infections to relieve the widespread emergence of antibiotic-resistant strains [106,107]. In an investigation of 1000 human non-antibiotic drugs, 27% of these drugs were confirmed to inhibit bacterial growth, and it was found that human target drugs have the same resistance mechanism as antibiotics, indicating the potential risk of promoting antibiotic resistance [34]. Non-antibiotic drugs can serve as efflux pump inhibitors to

act as adjuvants in antibiotic therapy, thus addressing efflux pump-mediated resistance. It has been demonstrated that chlorpromazine, amitriptyline and trans-clomipramine could reduce or reverse antibiotic resistance in various bacteria [108]. Through the overexpression profiling of the mutant *Escherichia coli tolC* strain, they confirmed the resistance to drugs using a combination of flow cytometry, whole-genome RNA sequencing and proteomics to test divalproex sodium, ethopropazine, methotrexate, niacinamide, tamoxifen and sulfadiazine [34]. Compared to antibiotics, non-antibiotic drugs are used more frequently and in a wider range of situations in both home and hospital care. Moreover, most drugs are not fully metabolized in the body and are excreted out of the body into the sewage system via feces and urine [109]. Consequently, thousands of non-antibiotic drugs that have not been metabolized properly end up in UWTPs, with unpredictable biological impacts on the microorganisms found in the activated sludge. Figure 1 shows the summarized concentration ranges of various non-antibiotic drugs in UWTPs influents and effluents reported in previous studies (details shown in Table S1). In recent years, various non-antibiotic pharmaceuticals have been detected in wastewater, including anticancer drugs, antidiabetics, antidiarrheals, antihistamines, anti-inflammatory drugs, antivirals, bronchodilators, cardiovascular drugs, diuretics, H_2 receptor agonists, hormone drugs, psychotropic drugs, scabicides and antipruritics, urinary tract drugs, and repellents, in which previous studies detected 0.1–3.0 μg/L ketoprofen [109], acetaminophen at 0.7–344 μg/L [109,110], 60–389 μg/L caffeine [110,111], 0.04–0.96 μg/L metoprolol [109], 0.04–1.4 μg/L N, N-diethylaniline [109], 0.06–2.5 μg/L carbamazepine [109,112], 0.8–0.7 μg/L domperidone [109], 0.03–2.8 μg/L benzotriazole [109], 0.01–0.2 μg/L gatifloxacin [110], 0.8–1.3 μg/L irbesartan [110], 16–19 μg/L valsartan [110], 3.6–9.2 μg/L metformin [110] and 0.2–4.1 μg/L fexofenadine [110].

The promotion of antibiotic resistance and horizontal gene transfer in bacteria has been partially validated by non-antibiotic drugs in previous studies [104,108,113–115]. Wang et al. [113] investigated the promotion of bacterial antibiotic resistance by non-steroidal anti-inflammatory drugs, ibuprofen, naproxen, diclofenac, lipid-lowering drugs, gefitinib, and β-blockers like propranolol. These drugs induced the overexpression of ARGs such as *marR*, *tetR*, *arcR*, *aceI*, and *ampC*. In addition, some non-antibiotic drugs could enhance bacterial resistance to specific antibiotics by promoting the expression of multidrug efflux pump systems. Laborda et al. [104] demonstrated that amitriptyline (an antidepressant), procaine (a local anesthetic), atropine (containing atropine), propranolol (a cardiovascular medication) and cimetidine (an antihistamine) could induce the expression of the multidrug resistance efflux pump system (MexCD-OprJ) of *Pseudomonas aeruginosa* to promote antibiotic resistance. However, ethionamide (an anti-tuberculosis drug) upregulates the multidrug resistance efflux pump system's (MexAB-OprM) gene expression. Antidepressants can enhance the production of reactive oxygen species, increasing the mutation frequencies, and also raise the selection for multiple antibiotic resistance to enrich resistant mutants [115]. Moreover, acetaminophen has been confirmed to enhance the conjugative transfer of ARGs via rising cell membranes, increasing reactive oxygen species (ROS) production, enhancing SOS response and upregulating conjugation bridges genes [116]. Taken together, these studies demonstrated that non-antibiotic drugs promoted antibiotic resistance through co-regulation, promoting mutation frequencies, and increasing conjugative frequencies

6. Microplastics

In recent years, studies have revealed a significant amount of microplastics in wastewater treatment systems [117–121]. Okoffo et al. [120] compiled the distribution of microplastics in wastewater across cities worldwide and found approximately 1 to 7000 microplastic particles per liter of water. Fragments and fibers ranging from 0.1 to 0.5 mm in size are common in wastewater. Among them, polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS) are the most common types of microplastics in wastewater [121,122]. Microplastics provide a favorable substrate for microbial attachment in aquatic environments, promoting the formation of biofilms. Zhang et al. [123] reported the enrichment of multi-antibiotic-resistant drug bacteria biofilms formed on the microplas-

tic surfaces compared to the surrounding water environment. Yang et al. [124] reanalyzed metagenomic data of marine microplastics and found the enrichment of metal and antibiotic resistance genes on microplastics. Wang et al. [125] used the quantitative PCR method to quantify the selective enrichment of ARGs and integrase genes in urban river microplastic biofilm. It is concerning that the microbial content carried by microplastics is significantly higher (100–5000 times) than that in the surrounding water environment [123]. In the exploration of extracellular DNA transformation in a microcosm, microplastic biofilms, including polypropylene (PP), polyethylene (PE), and polystyrene (PS), showed an extreme transformation ability that was a thousand-fold greater than the natural substrate, i.e., quartz sands with 300 μm and 3 mm diameters [126]. Furthermore, the small-size microplastics and the aged microplastics further presented higher transformation rates than large-size microplastics and pristine microplastics, indicating that the interface and size characteristics were the main drivers for promoting extracellular DNA transformation on microplastic biofilms. Based on gene expression levels, this study confirmed the overexpression of flagellum movement-related genes (*mot*A and *pga*A) and DNA translocation regulatory genes (*pil*X and *com*A), which facilitated biofilm formation to enhance bacterial cell density and synthesized more pili fixed on the bacterial cell surface to catch more extracellular DNA. Yu et al.'s (2023) study proved conjugative transfer was another method for promoting the horizontal gene transfer of ARGs [127]. Therefore, the presence of microplastics in wastewater greatly increases the burden of ARB and ARGs in aquatic environments.

To add insult to injury, the adsorptive properties of microplastics determine their ability to strongly accumulate heavy metals, antibiotics, and other organic pollutants, thereby creating a microecological environment where microplastics serve as a core, pollutants act as substrates and microbials attach to biofilms [128–133]. Yu et al. [133] investigated the adsorption of heavy metal ions and antibiotics onto microplastic surfaces. This study demonstrated that different heavy metal ions in aquatic environments can either enhance or inhibit the adsorption of antibiotics on microplastic surfaces. It also suggested that the adsorption of antibiotics and heavy metal ions on microplastic surfaces can undergo dynamic changes in various water environments. Imran et al. [37] reported heavy metals adsorbed on microplastics' surfaces to create a high concentration of metal microenvironments, and led to the enrichment of ARB in the biofilm formation on microplastics through the co-selective effects of heavy metals. However, the biofilm formed on microplastics could also influence the adsorption of pollutants, particularly enhancing the adsorption of hydrophobic organic compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Wang et al. [134] found that the triclosan disinfectant adsorbed by microplastics promoted the enrichment of ARGs in surface-colonized biofilms. Recently, a combination experiment of antibiotics, triclocarban, and (or) heavy metals with microplastics showed that copper-absorbed polyethylene microplastics (PE MPs), ampicillin-absorbed PE MPs, and triclocarban-absorbed PE MPs showed a higher ARG level (the total of 22 ARGs) than single-exposure to each other [135]. Copper–ampicillin-absorbed PE MPs, triclocarban–ampicillin-absorbed PE MPs and triclocarban–copper-absorbed PE MPs further exhibited a higher ARG level than copper-absorbed PE MPs, ampicillin-absorbed PE MPs and triclocarban-absorbed PE MPs. This result shows that microplastics not only acted as bacterial colonizers, but also enriched other ARDs, leading to a huge increase in microbial antibiotic resistance. A new problem has come to light related to the effects of ARDs in combination (synergistic, additive, antagonistic), and especially their microplastic-centered joint effects.

7. Mechanisms for Promoting Antibiotic Resistance in Bacteria

The promotion of bacterial antibiotic resistance by ARDs in urban wastewater was typically achieved through enhancing co-selection pressure, increasing mutation frequencies, promoting HGT, and aggregating ARDs with colonized biofilms (Figure 2). The co-selection of ARDs, like heavy metals, primarily included three mechanisms: co-resistance, cross-

resistance, and co-regulation [30]. Cross-resistance refers to simultaneous resistance to antibiotics and other pollutants [30]. For example, multi-antibiotic efflux pump systems in *Listeria monocytogenes* could discharge intracellular metals at the same time [73,136]. Co-resistance refers to ARGs and other pollutant-tolerated genes both located on the same genetic element [75], in which the host is stressed by pollutants and ARGs would enrich during host reproduction. Research has shown that plenty of ARGs and metal resistance genes were co-located on plasmids, which could exhibit co-resistance to metals and antibiotics [137]. The co-regulation of resistance implies that other pollutant resistance genes and ARGs share a common regulatory system [30,136], in which pollutants simultaneously activate resistance to other pollutants and antibiotics. Specifically, the czcR-czcS regulatory system in *Pseudomonas aeruginosa* can simultaneously regulate the antibiotic efflux pump system OprD and the cobalt–zinc–cadmium efflux system CzcCBA [76]. Moreover, the increasing mutation frequencies were also relevant mechanisms in promoting antibiotic resistance through increasing resistance variability. ARDs usually increase the production of bacterial intracellular reactive oxygen species and stimulate SOS response to induce DNA repair and mutagenesis [115]. The mutation frequency of the test strains exposed to 1 ng/L metformin for 1 day was increased at least 1.98- to 4.46-fold compared to the control, in which some gene mutagenesis increased multiple antibiotic resistance by driving regulatory genes to increase efflux systems [138]. Increasing studies revealed that the promotion of HGT was the main mechanism of ARDs' increasing antibiotic resistance, which leads to increasing cell membrane permeability, increasing the chance of cell-to-cell contact, the overexpression of outer membrane proteins, the increasing expression of horizontal transfer genes, stimulating SOS response, and changing the intracellular metabolite levels to enhance plasmid transmission. Besides this, microplastic carriers could reproduce ARB and enrich ARGs on surfaces and interspaces, and then adsorb ARDs to further directly and indirectly facilitate antibiotic resistance.

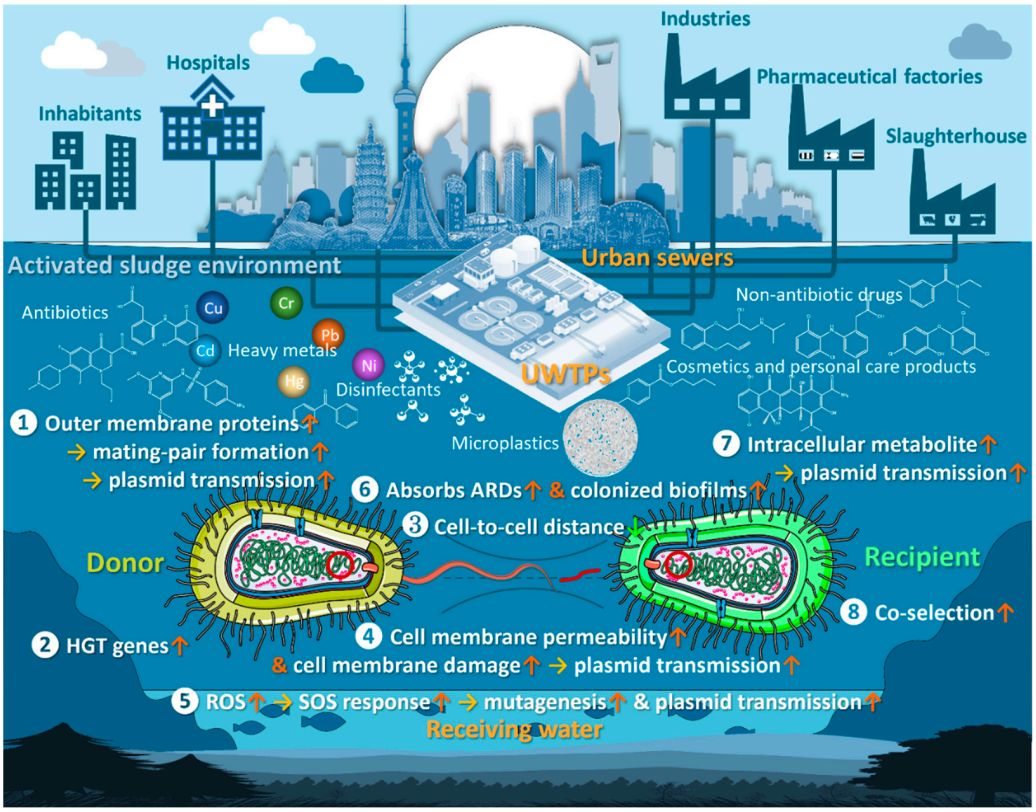


Figure 2. Mechanisms of antibiotic resistance drivers in activated sludge environments from urban wastewater treatment plants. Horizontal arrows (yellow) indicate causality; upward arrows (orange) indicate increasing trends; downward arrows (green) indicate decreasing trends.

8. Current Bottleneck and Perspectives

UWTPs have attracted significant attention as a critical environmental node for controlling ARGs and ARB. Research has gradually been conducted on ARDs and their mechanisms in recent years. Meanwhile, UWTPs serve as reservoirs for ARGs, ARBs, and various chemical pollutants. The impact of these chemical pollutants on ARGs and ARBs, particularly their role in promoting bacterial antibiotic resistance, should not be overlooked. Currently, studies on ARDs and their mechanisms have been focused on single-contaminant exposure experiments in the laboratory with single strains. However, in the actual activated sludge environment, the microbial community (suspended, flocculent and biofilm) is exposed to the composite stress of the pollutants. In the future, to effectively reduce the impact of bacterial antibiotic resistance in downstream environments, it is essential to strengthen the coordinated control of pollutants that promote bacterial antibiotic resistance, in order to further control ARGs and ARBs in wastewater treatment systems. It is crucial to limit bacterial resistance and safeguard human health and ecological safety by thoroughly blocking the circulation of ARGs and ARBs in wastewater treatment systems.

The following aspects are recommended for further study:

To conduct ARD exposure experiments at actual concentration levels consistent with the activated sludge environment.
To evaluate the effects of long-term exposure to ARDs on strains.
To reveal the mechanisms of joint antibiotic resistance promotion and the joint effects of ARDs in combination (synergistic, additive, antagonistic).
To investigate the mechanisms of promoting antibiotic resistance to activated sludge microbiomes.
To develop a high-throughput screening technique and assessment system for finding the mechanism promoting antibiotic resistance in numerous unassessed pollutants.
To establish a quantitative risk assessment system for promoting antibiotic resistance to contaminants.
To develop ARG-targeted control technology for the activated sludge microbiome in UWTPs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w15173165/s1, Table S1: Detailed data on concentrations of antibiotic resistance drivers in urban wastewater treatment plants collected in previous studies. References [51–58,82,109–112] are cited in the Supplementary Materials.

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