Chapter

The Efficiency of Wastewater Treatment Plants for the Removal of Antibiotics

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Abstract

Undoubtedly domestic Wastewater Treatment Plants (WWTPs) are not designed for the removal of some pollutants such as antibiotics. This chapter summarizes the occurrence and fate of six groups of the most widely used antibiotics (β-lactams, sulfonamides, quinolones, tetracyclines, macrolides, and others) in domestic WWTPs. The literature showed that the six groups of antibiotics have been frequently detected during wastewater treatment train (influent, primary treatment, secondary treatment, tertiary treatment, effluent, and sludge treatment) of domestic WWTPs. Also, it was clear that the main removal routes of antibiotics during sewage treatment of domestic WWTPs were adsorption, biodegradation, membrane filtration, and disinfection. Domestic WWTPs cannot remove most of the antibiotics which finally enter the environment through treated effluent and sludge.

Keywords: antibiotics, adsorption, biodegradation, domestic wastewater, organic pollutants, wastewater treatment plants

1. Introduction

Nowadays, environmental researchers have extended their focus beyond classic environmental contaminants such as pesticides, Polychlorinated Biphenyls (PCBs), and dioxins [1, 2]. Antibiotics are one of the new serious environmental contaminants. Antibiotics, a group of pharmaceuticals used as a medicine and growth promoter for both humans and animals, are considered a new serious environmental contaminant due to their continuous input into the environment and persistent presence [3–5].

Antibiotics are chemicals classified depending on their nature into three main categories: natural, semi-synthetic, and synthetic. Moreover, antibiotics can be classified depending on their mode of action into two categories: bactericidal (kill microorganisms) and bacteriostatic (impede microbial growth).

In developing countries, there is an increase in the consumption of antibiotics, without any prescription from physicians, to cure the different kinds of diseases that originate from improper general hygiene and poor sanitation systems. Also, antibiotics are used widely in animal farming for the protection of animal health to maintain the high demand for animal products [6].

Boyles et al. [7] reported that the high consumption of antibiotics by humans (households, hospitals, and industry) and in veterinary results in the increasing release of unchanged active ingredients and partially metabolized antibiotics into the sewer system (directly or indirectly) which consequently reach into the domestic wastewater treatment plants [8–10].

Conventional wastewater treatment plants cannot completely remove antibiotics and these antibiotics will finally contaminate the environment through effluent or sludge [11, 12]. Therefore, local wastewater treatment plants act as one of the main pathways for antibiotics to transfer into the environment [13].

Although antibiotics residues in water are very low (ng/L to μg/L), they still draw the researchers' attention in the whole world since these antibiotics are the main source for the occurrence and transfer of Antibiotic-Resistant Genes (ARG) and Antibiotic-Resistant Bacteria (ARB) which have serious impacts on the environment [14, 15].

During last years, simultaneous detection of trace concentrations of antibiotics in wastewater and sludge samples is no longer difficult as a result of the invention of new detection methods such as liquid chromatography-mass spectrometry, solid-phase extraction, and ultra-performance liquid chromatography-mass spectrometry with the rapid development of analytical methods such as Solid Phase Extraction (SPE), High-Performance Liquid Chromatography Mass Spectrometry (HPLC-MS/MS) and Ultra-Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS) [16, 17].

The occurrence of antibiotics in water environments such as groundwater and surface water was summarized by previous studies [18–22] as well as sediments, sludge, and soil [19]. Nevertheless, most of these studies focused only on the occurrence of antibiotics in different environments with little focus on the fate of antibiotics. For example, summarizing the removal of antibiotics in sediment, water, and soil environments, instead of the elimination of antibiotics in wastewater treatment plants [21]. Also, the elimination of Personal Care Products (PPCPs) and pharmaceuticals via biodegradation and other pathways in wastewater treatment plants, with limited content on antibiotics elimination [23].

Thus, in this review, we summarize the data and information on the occurrence and fate of antibiotics in wastewater treatment plants (WWTPs) to provide the overall profile of antibiotics concentrations in influent, treatment stages, sludge, and effluent of wastewater treatment plants, and to understand the elimination routes and fate of antibiotics in WWTPs.

2. Occurrence of antibiotics in the aquatic environment

Domestic wastewater treatment plants receive most of the used antibiotics through the sewer network, while the rest of the antibiotics are dumped directly into rivers and streams or escape as leachate from landfills. **Figure 1** summarizes the introduction pathways of antibiotics into the aquatic environment.

3. Occurrence of antibiotics in wastewater treatment plants (WWTPs)

Antibiotics can be classified using different ways such as their chemical structure. According to the chemical structure, there are 12 different classes of antibiotics (**Table 1**) such as β- lactams, aminoglycosides, macrolides, glycopeptides,

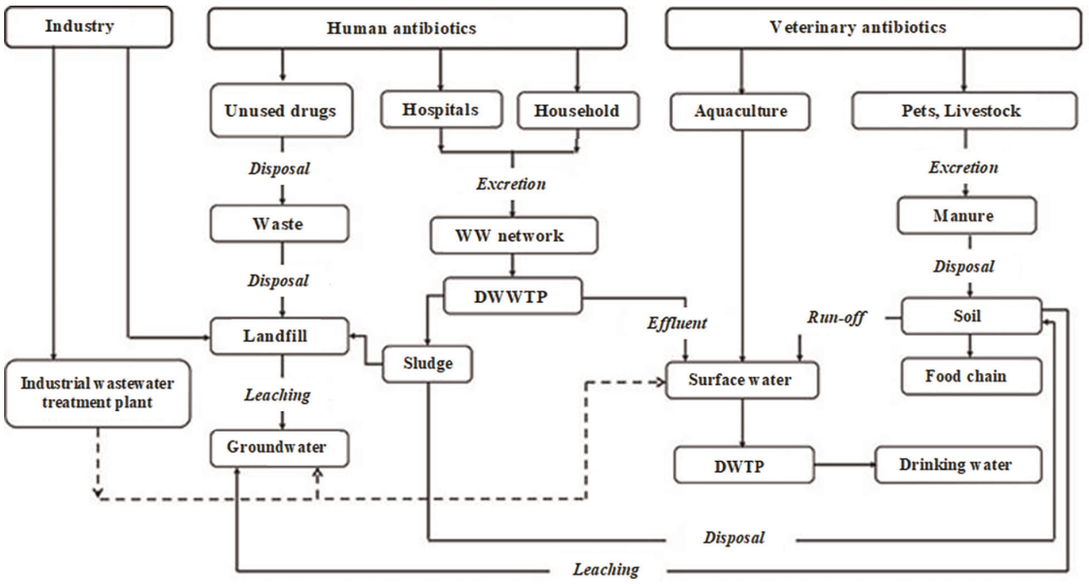


Figure 1.
Pathways of antibiotics in the aquatic environment, WWTP: Wastewater treatment plant, DWTP: Drinking water treatment plant.

oxazolidinones, sulfonamides, quinolones (fluoroquinolones), polymyxins, tetracyclines, streptogramins, and others such as chloramphenicol, thiamphenicol, lincomycin, trimethoprim, and clindamycin [5, 8]. **Table 1** summarizes the chemical structure of the different 12 classes of antibiotics and their mode of action, mechanism of action as well as discovery dates.

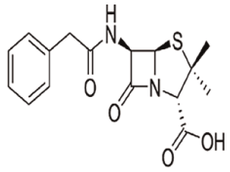
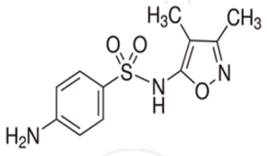
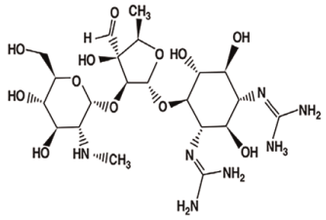
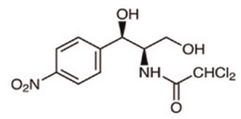
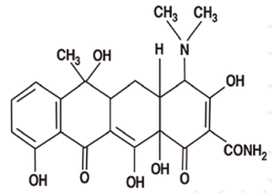
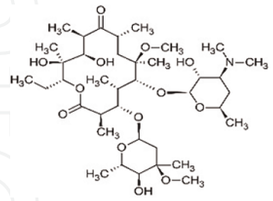
In this review, **Tables 2**–**7** summarize data about the occurrence of the most common antibiotics (6 classes) in influent and effluent samples of wastewater treatment plants. These antibiotics include β-lactams, quinolones, sulfonamides, macrolides, tetracyclines, and others, while **Table 8** summarizes the occurrence of these antibiotics in sludge generated from wastewater treatment plants.

Figure 2 shows the typical structure and the treatment train of a domestic wastewater treatment plant. The treatment train begins consists of three stages including primary, secondary, and tertiary. The primary treatment stage contains preliminary treatment units (screens and sand & grit removal), an oil and grease removal unit, and primary settling tanks (not common in most WWTPs). The secondary treatment stage contains a biological treatment unit (aeration tank) using different methods such as activated sludge (AS) and trickling filters (TF), followed by secondary settling tanks (secondary clarifiers). The tertiary treatment stage contains a sand filtration unit followed by a disinfection unit using different technologies such as chlorination, ozonation, and ultraviolet (UV), and finally nutrient removal unit (for treated effluent which contains high concentrations of phosphorus and nitrogen).

3.1 Presence of antibiotics in influent and effluent samples of WWTPs

3.1.1 β-Lactams

β-lactams are a group of antibiotics characterized by the presence of the β-lactam ring. The β-lactams ring is the main structure that gives the antibacterial activity

Antibiotic group	β-Lactams	Sulfonamides	Aminoglycosides	Chloramphenicol	Tetracyclines	Macrolides
Chemical structure						
Main feature	All contain a beta-lactam ring.	All contain the sulfonamide group	All contain aminosugar substructures	Distinct individual compound (shown)	All contain 4 adjacent cyclic hydrocarbon rings	All contain a 14-, 15-, or 16-membered macrolide ring
Examples	• Penicillins (penicillin G shown) such as flucloxacillin and amoxicillin. • Cephalosporins such as cefalexin.	Sulfisoxazole (shown), sulfanilamide, prontosil	Streptomycin (shown), kanamycin, neomycin		Tetracycline (shown), oxytetracycline, limecycline	Clarithromycin (shown), erythromycin, azithromycin
Mode of action	Bactericidal	Bacteriostatic	Bactericidal	Bacteriostatic	Bacteriostatic	Bacteriostatic
Mechanism of action	Inhibit bacterial cell wall synthesis	Inhibit bacterial growth and multiplication	Inhibit bacterial protein synthesis	Inhibit bacterial protein synthesis	Inhibit bacterial protein synthesis	Inhibit bacterial protein synthesis
Discovery	1920s	1935	1943	1947	1948	1950s

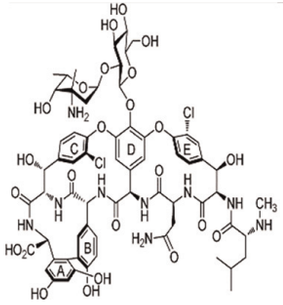
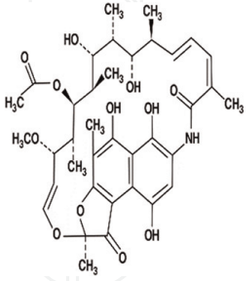
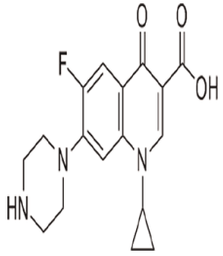
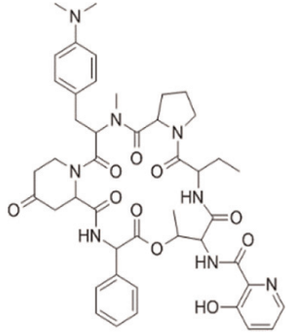
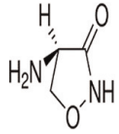
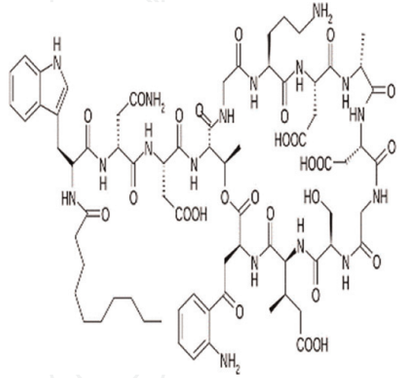
Antibiotic group	Glycopeptides	Ansamycins	Quinolones	Streptogramins	Oxazolidinones	Lipopeptides
Chemical structure						
Main feature	Consist of carbohydrate linked to a peptide formed of amino acids	All contain an aromatic ring bridged by an aliphatic chain	All contain fused aromatic rings with an attached carboxylic acid group	Combination of two structurally different compounds	All contain 2-oxazolidone somewhere in their structure	All contain a lipid bonded to a peptide
Examples	Vancomycin (shown), teicoplanin	Rifamycin (shown), geldanamycin	Ciprofloxacin (shown), trovafloxacin	Pristinamycin IA (shown), Pristinamycin IIA	Cycloserine (shown), linezolid	Daptomycin (shown), surfactin
Mode of action	Bactericidal	Bactericidal	Bactericidal	Bactericidal	Bacteriostatic	Bactericidal
Mechanism of action	Inhibit bacterial cell wall synthesis	Inhibit bacterial RNA synthesis	Inhibit bacterial DNA replication and transcription	Inhibit bacterial protein synthesis.	Inhibit bacterial protein synthesis	Disturb multiple cell membrane functions
Discovery	1958	1959	1962	1962	1978	1986

Table 1.
Different classes of antibiotics and their mode of action and mechanism of action.

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Penicillin G	29.0	NA	Grab	Primary treatment
	ND-10	ND-300	Composite	—
Penicillin V	20–13,800	ND–2000	Grab	Primary treatment
Amoxicillin	190–280	ND–30	24 h FPC	Membrane filtration/Reverse osmosis
	1400–6940	ND-50	Composite	—
Ampicillin	NA	7	Grab	Activated sludge
Oxacillin	NA	<20	24 h FPC	Activated sludge + chlorination
Cloxacillin	ND–320	ND	Grab	Activated sludge
	ND-4600	ND-700	Composite	—
Cefalexin	1200	980	Grab	Activated sludge + denitrification and nitrification
	NA	1110–1410	24 h FPC	Chemically enhanced primary treatment
	2800–64,000	ND-250	Composite	—
Cefotaxime	24	<12	Grab	Activated sludge + denitrification and nitrification
Cefaclor	500–980	ND–60	Grab	Activated sludge
	500–6150	ND-1800	Composite	—
Cefradine	NA	12	Grab	Activated sludge
Cefazolin	NA	27	Grab	Activated sludge

ND: Not detected, NA: Not analyzed, FPC: Flow proportional composite.

Table 2.
Concentrations of β-lactams in wastewater.*

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Sulfamethoxazole	360–500	270–320	Grab	Activated sludge
	255–333	ND	Grab	Membrane filtration/Reverse osmosis
	<80–674	<80–304	1-week FPC	Activated sludge + denitrification
	390	310	48 h composite	—
	230–750	211–860	24 h FPC	Activated sludge + sand filtration
N^4-acetylsulfamethoxazole	850–1600	<20–180	72 h FPC	Activated sludge + sand filtration
	NA	690–2200	Grab	—
Sulfathiazole	2–40	ND–5	Composite	Activated sludge
Sulfasalazine	ND–60	ND–10	Grab	Activated sludge
	ND-100	4–150	Composite	—
Sulphapyridine	60–150	40–350	24 h FPC	Activated sludge + sand filtration
	NA	124	24 h composite	—
	NA	85–88	27 h FPC	Activated sludge + sand filtration

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Sulfacetamide	NA	64–151	24 h FPC	Activated sludge + chlorination
	110–210	ND	24 h composite	Activated sludge
Sulfacholoropyridazine	<30–476	<30–149	Grab	Activated sludge
Sulfadiazine	3.7	1.9–3.8	Grab	Activated sludge
Sulfisoxazole	NA	0.13	Grab	Activated sludge
Sulfadimethoxine	<10–213	<10 – 70	Grab	Activated sludge
Sulfamethizole	ND–710	ND–10	Grab	—
Sulfamerazine	29–73	12–42	Grab	—
Sulfanilamide	NA	26	Grab	Activated sludge
Sulfaguanidine	NA	2	Grab	—

**ND: Not detected, NA: Not analyzed, FPC: Flow proportional composite.*

Table 3.
Concentrations of sulfonamides in wastewater.*

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Norfloxacin	110	85	Grab	Activated sludge + denitrification and nitrification
	339	85	24 h composite	Activated sludge
	72–174	7–37	1-week FPC	Activated sludge + denitrification
	NA	250–546	24 h FPC	Chemically enhanced primary treatment
Ciprofloxacin	3800–4600	640–720	Grab	Activated sludge
	210	60	24 h composite	Activated sludge
	113–300	7–32	1-week FPC	Activated sludge + denitrification
Enrofloxacin	10–100	10	Grab	Activated sludge
	ND-40	2–50	Composite	—
Nalidixic acid	ND–200	55	Grab	Activated sludge
Ofloxacin	137	41	Grab	Activated sludge + chlorination
	ND-200	ND-450	Composite	—
	470	110	48 h composite	—
	NA	740–1220	24 h FPC	Chemically enhanced primary treatment
Lomefloxacin	NA	<45	Grab	Activated sludge + chlorination + sand filtration
Flumequine	NA	15	Grab	Activated sludge
Pipemidic acid	NA	70	Grab	—
	54	12	24 h composite	—
Flerofloxacin	28	5.8	24 h composite	Activated sludge

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Lomefloxacin	98	17	24 h composite	—
Gatifloxacin	111	56	24 h composite	—
Moxifloxacin	44	17	24 h composite	—

*ND: Not detected, NA: Not analyzed.

Table 4.
Concentrations of quinolones in wastewater.*

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Tetracycline	240–790	50–160	24 h composite	Activated sludge
	96	180	24 h FPC	Activated sludge + denitrification and nitrification
	NA	683–1420	Grab	Primary treatment
Oxytetracycline	NA	<5.0–100	24 h FPC	Chemically enhanced primary treatment
	ND	ND–20	Grab	Activated sludge
Chlortetracycline	10–35	ND	Grab	Membrane filtration/Reverse osmosis
	270	60	24 h FPC	Activated sludge + chlorination
Demeclocycline	50	ND	Grab	—
Doxycycline	ND–65	ND–40	Grab	Activated sludge
	20–650	10–150	Composite	—
	<64–2210	<64–915	1-week FPC	Activated sludge + denitrification

*ND: Not detected, NA: Not analyzed, FPC: Flow proportional composite.

Table 5.
Concentrations of tetracyclines in wastewater.*

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Erythromycin-H_2O	810	850	Grab	Primary treatment
	470	520	Grab	Activated sludge +DN
	NA	105	24 h composite	—
	60–190	60–110	24 h FPC	Activated sludge + sand filtration
	NA	1310–4330	Grab	Primary treatment
Erythromycin	71–141	145–290	24 h FPC	Trickling filters + activated sludge + UV
	830	620	24 h FPC	Activated sludge + denitrification

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Tylosin	ND–55	ND–65	Grab	Activated sludge
	20–40	1–5	Grab	Membrane filtration/Reverse osmosis
	ND-60	3–3400	Composite	—
	1150	60	24 h composite	—
Roxithromycin	ND–18	ND–100	Grab	Activated sludge
	140–175	10–15	Grab	Membrane filtration/Reverse osmosis
	10–40	10–30	24 h FPC	Activated sludge + sand filtration
	75	35	24 h composite	Activated sludge + denitrification
Oleandomycin	20–190	5–30	Grab	Membrane filtration/Reverse osmosis
	ND-5	ND-150	Composite	—
Azithromycin	6–53	9–28	Grab	Oxidation ditch + chlorination
	90–380	80–400	24 h FPC	Activated sludge + sand filtration
Clarithromycin	NA	172	Grab	Activated sludge
	NA	57–328	24 h FPC	Activated sludge + sand filtration
	NA	220–329	27 h FPC	Activated sludge + sand filtration

[*]ND: Not detected, NA: Not analyzed, FPC: Flow proportional composite.

Table 6.
Concentrations of macrolides in wastewater[*].

Antibiotic	Concentration (ng/L)		Sampling type	Treatment technology
	Influent	Effluent		
Trimethoprim	213–300	218–322	Grab	Trickling filters + activated sludge + UV
	7900	2400	Grab	Activated sludge + sand filtration
	NA	68–81	72 h FPC	Activated sludge + sand filtration
	210–440	20–310	24 h FPC	Activated sludge + sand filtration
	140–1300	66–700	1-week FPC	Activated sludge + denitrification
Chloramphenicol	31	17	24 h FPC	Oxidation ditch + UV
	NA	92–1050	Grab	Primary treatment
	NA	50	24 h FPC	Activated sludge
Thiamphenicol	NA	138	Grab	Activated sludge
Clindamycin	2–5	5	Grab	Activated sludge
	20–60	5–70	Composite	—
	1–10	ND–5	Grab	Membrane filtration/Reverse osmosis
Lincomycin	60–80	50–60	Grab	Activated sludge
	20–500	3–30	Composite	—

[*]ND: Not detected, FPC: Flow proportional composite.

Table 7.
Concentrations of others in wastewater[*].

Antibiotic	Concentration (ng/L)		Treatment technology	Country or region
	Influent	Effluent		
Sulfonamides				
Sulphapyridine	28	1	Activated sludge + sand filtration	Switzerland
Sulfamethoxazole	20	NA	Oxidation ditch + UV	China
Sulfadimidine	31	NA	Oxidation ditch + UV	China
Sulfadimethoxine	<2.0–8.1	NA	—	USA
Sulfisoxazole	<4.1–21.9	NA	—	USA
Macrolides				
Roxithromycin	61–131	NA	Activated sludge + denitrification and nitrification	Switzerland and Germany
Azithromycin	52–158	2.3	Activated sludge + denitrification and nitrification	Switzerland and Germany
Clarithromycin	27–63	0.8	Activated sludge + denitrification and nitrification	Switzerland and Germany
Erythromycin-H_2O	76	NA	Activated sludge + chlorination	China
Quinolones				
Ofloxacin	886	NA	Oxidation ditch + UV	China
Norfloxacin	301	NA	Activated sludge + chlorination	China
Ciprofloxacin	NA	1400–4800	Activated sludge + denitrification and nitrification	Sweden
Tetracyclines				
Tetracycline	<7.5–15.8	NA	—	USA
Chlortetracycline	<6.9–14.7	NA	—	USA
Doxycycline	NA	1300–1500	Activated sludge + denitrification and nitrification	Sweden
Others				
Clindamycin	3.7–15.4	NA	—	USA
Trimethoprim	21–133	0.1	Activated sludge + denitrification and nitrification	Switzerland and Germany

*NA: Not analyzed.

Table 8.
Concentrations of antibiotics in sludge.*

for β-lactams. The variation in pharmacological properties depends on the differentiation in the side chains. β-lactams include two subclasses which are cephalosporins and penicillins [24].

The β-lactam antibiotics industry has annual sales of about $15 billion [25]; this makes up 65% of the total antibiotics market [26]. 6-Aminopenicillanic acid is the precursor to produce β-lactam antibiotics, which can be produced using free and immobilized penicillin G acylase [27]. Immobilized enzymes are preferred over the free enzyme for many reasons including product-free enzymes as reported by Elnashar [28].

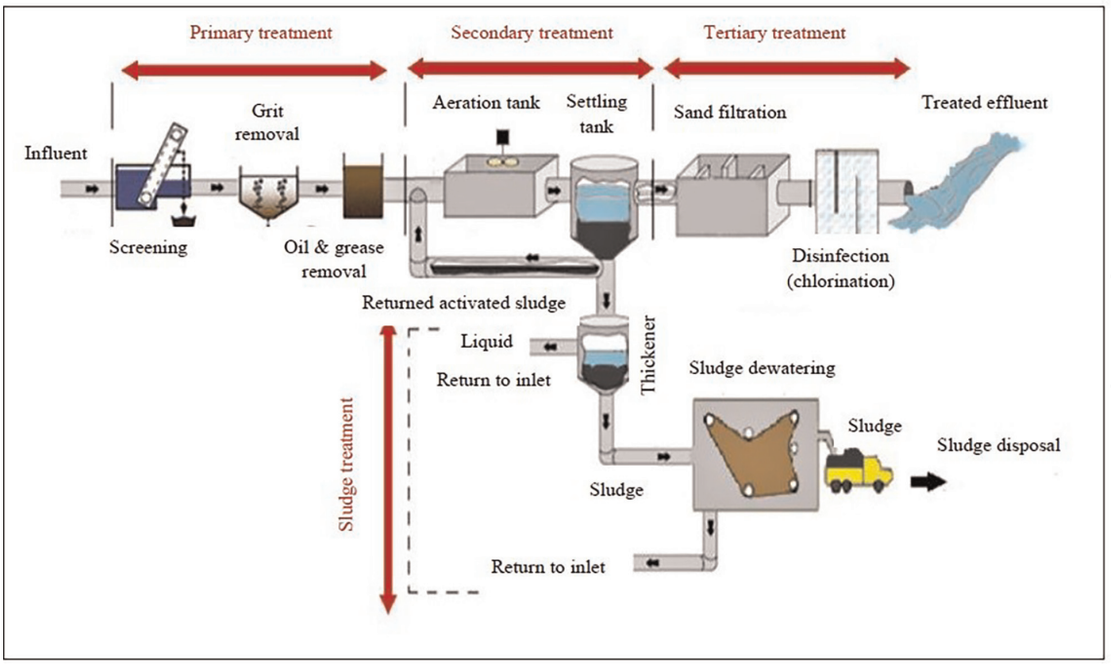


Figure 2.
Typical structure of domestic wastewater treatment plant.

In the samples collected from WWTPs (**Table 2**), some penicillins were detected such as ampicillin, penicillin G & V, amoxicillin, cloxacillin, and oxacillin. Penicillin V showed the highest concentrations (2000–2013,800 ng/L) in influent and effluent wastewater samples [29]. For cephalosporins, six types were detected including cefotaxime, cefalexin, cefaclor, cloxacillin, cefazolin, and cephradine [5, 30]. Generally, β-lactams, especially penicillins were rarely detected in domestic wastewater though they are used in great amounts [14, 31]. This may be attributed to the unstable nature of the β-lactam ring. The β-lactam ring can be broken by a popular bacterial enzyme group called β-lactamases [22] or can be broken through the chemical hydrolysis process [32].

3.1.2 Sulfonamides

Sulfonamides are used commonly since 1968 and consist of a large group of broad-spectrum antibiotics [5, 13, 33]. In WWTPs, 16 types of sulfonamides have been detected (**Table 3**). The most detected sulfonamides were sulfamethoxazole (5597–6000 ng/L) followed by sulfamethazine, sulphapyridine, and sulfadiazine, sequentially. N^4-acetylsulfamethoxazole and many other N^4-acetylated sulfonamides, dominant human metabolites of sulfonamides, were also detected in WWTPs and it was discovered that these metabolites can be transformed into their parent compounds [11, 34, 35].

3.1.3 Quinolones (fluoroquinolones)

Quinolones are a class of antibiotics characterized by the presence of quinolone branches as their basic structure. Fluoroquinolones are a subclass of quinolones that contains a fluorine-substituted central ring. Nalidixic acid was the first quinolone antibiotic discovered in the 1960s followed by newly developed four generations of

quinolones [3, 35]. As a result of the universal extensive usage of quinolones, all generations of these antibiotics were detected worldwide in WWTPs as shown in **Table 4**. The most commonly detected quinolones in WWTPs were ciprofloxacin, norfloxacin, and ofloxacin [8, 12, 36]. The highest concentrations detected in influent and effluent samples were 4600 and 7870 ng/L for ciprofloxacin and ofloxacin, respectively [11, 37, 38].

3.1.4 Tetracyclines

Tetracyclines are composed of eight antibiotics (semisynthetic and natural) which inhibit the synthesis of bacterial proteins. Tetracyclines are widely used for human use, the poultry industry, and animal agriculture [39]. In WWTPs, five types of tetracyclines were detected in influent and effluent samples and tetracycline was the most detected one as shown in **Table 5** [5, 14]. Doxycycline showed the highest concentration (2210 ng/L) in influent samples [40], while tetracycline showed the highest concentration (1420 ng/L) in effluent samples [37]. Tetracyclines were discovered in sludge samples with no or little biodegradation. Generally, as shown in **Table 8**, tetracyclines have a relatively rare presence in WWTPs because tetracyclines are used rarely by humans [41, 42].

3.1.5 Macrolides

Macrolides are a group of antibiotics characterized by the presence of a lactone ring that is substituted with alkyl, hydroxyl, and ketone groups, which inhibit the synthesis of bacterial proteins and are usually used as substitutes for penicillin [1, 34]. In WWTPs, six types of macrolides and one metabolite of erythromycin (erythromycin-H_2O) were detected in influent and effluent samples. The most frequently detected macrolide was erythromycin-H_2O followed by roxithromycin, clarithromycin, azithromycin, and tylosin in sequence as shown in **Table 6**. The lowest detected macrolides in sequence were erythromycin-H_2O, roxithromycin, clarithromycin, azithromycin, and tylosin. Erythromycin-H_2O showed the highest concentrations in the influent (10,025 mg/L) and in the effluent (4330 ng/L) wastewater samples [37].

3.1.6 Others

In this review, the other category of antibiotics consists of five different types including chloramphenicol, trimethoprim, lincomycin, clindamycin, and thiamphenicol. All these other antibiotics were detected in WWTPs, and trimethoprim was the most abundant and widely distributed one as shown in **Table 7**. Trimethoprim showed the highest concentrations in influent (7900 ng/L) and in effluent (3052 ng/L) samples, while Chloramphenicol and thiamphenicol showed the lowest concentrations (<4 to 1050 ng/L) in WWTPs [14, 41].

3.1.7 Summary

Despite that β-lactams are the most consumed antibiotic by humans, they were not detected frequently due to the unstable nature of β-lactams [21]. Many reasons affecting the significant variation of antibiotics' concentrations in wastewater influents such as:

a. the consumption pattern of antibiotics, which may be different in different countries and even in the same country there is a great variation in consumption patterns of antibiotics [21].

b. different sampling procedures; grab/composite sampling results in a great variation in antibiotic concentrations since pharmaceutical loads reached WWTPs are different during the day [43].

c. hourly and seasonal fluctuation; seasonal variation of antibiotic consumption was high between summer and winter since antibiotic consumption is two times higher than consumption in summer which affects the concentrations of antibiotics in wastewater influent samples [44, 45]. Also, during winter, antibiotics in influent samples are diluted by rain [11, 46].

d. wastewater treatment plant scale, which means that higher concentrations of antibiotics in influent samples occurred when WWTP serves a low population and vice versa. Additionally, the concentrations of antibiotics varied in effluent depending on the different sampling methods and the applied wastewater treatment technology [5, 8].

3.2 Presence of antibiotics in sludge samples of WWTP

The analysis process for antibiotics and their transformations in sludge samples is a challenge since antibiotics in sludge have a low detectability rate as well as a low extraction pattern [47]. Therefore, the studies on the detection and presence of antibiotics in sludge are much less than those on wastewater [48]. The results showed the presence of five different classes of antibiotics in both activated and digested sludge samples as shown in **Table 8**.

The most abundant antibiotics were ciprofloxacin (4.8 mg/kg) in digested sludge [40] and norfloxacin (2.7 mg/kg) in activated sludge [14, 49]. Quinolones were detected mostly at the level mg/kg, while most tetracyclines, macrolides, sulfonamides, and others were detected in lower levels (μg/kg) [11]. Despite the wide usage of β-lactams antibiotics in veterinary and human medicine, all sludge samples all over the world showed no presence of β-lactams antibiotics which is mainly attributed to their poor adsorption onto sludge and their unstable characteristics [37].

4. Antibiotics transformation and fate in WWTPs

4.1 Antibiotics removal pathways in WWTPs

Antibiotics in WWTPs can be removed through major pathways including biodegradation, adsorption, membrane separation, and disinfection. Furthermore, there are other pathways for the removal of antibiotics including photolysis, volatilization, and hydrolysis which were eliminated since they have an inconsiderable role in the reduction of antibiotics from WWTPs. For example, β- lactams antibiotics are not stable because of the presence of β- lactam ring which easily can be hydrolyzed. Thus, β-lactams will be hydrolyzed before reaching the WWTPs.

Additionally, some researchers reported that β- lactams have a relatively long half-life due to hydrolysis at neutral pH values (same as in WWTPs) such as more than

5 days for amoxicillin [50] and 52 h for meropenem [51]. Moreover, although β-lactams can be hydrolyzed in WWTP, the contribution of the hydrolysis process for the removal of antibiotics is useless because the wastewater treatment process has a relatively short hydraulic retention time (8–20 h).

In addition to that, there are some antibiotics such as amoxicillin which were degraded by sunlight-photolysis or UV-photolysis [50], macrolides [52], quinolones [53], and tetracyclines [54, 55]. However, this degradation process has a minor significance because wastewater has high concentrations of suspended solids which inhibit the deep penetration of sunlight or UV [5, 8]. Besides, the effect of the photolysis process can be neglected since the hydraulic retention time of WWTPs is much lower than the half-life of most antibiotics in wastewater.

4.2 Antibiotics transformation and fate in conventional WWTP

4.2.1 Primary treatment units

The primary treatment stage in WWTPs consists mainly of screens and primary settling tanks. In some WWTPs, some coagulant chemicals are added in primary treatment units such as ferric ion salts, aluminum salts, or polymers called CEPT (Chemically Enhanced Primary Treatment) [56]. Many previous studies reported that the primary treatment stage in WWTPs has no significant removal for different types of antibiotics including clarithromycin, sulfamethoxazole, ofloxacin, cefalexin, erythromycin, amoxicillin, clindamycin, and cefaclor [13].

Nevertheless, other studies reported that the chemically enhanced primary treatment (CEPT) process can significantly improve the removal efficiencies of some antibiotics such as norfloxacin (67.7%), ofloxacin (55.2%), erythromycin (44.8%), sulfamethoxazole (64.0%), and roxithromycin (76.3%). This effect is due to the destroying effect of coagulants on the chemical chains of some antibiotics [11].

4.2.2 Biological treatment units

Clearly, in biological treatment units, biodegradation and adsorption processes are the main pathways for the transformation of antibiotics in WWTPs. According to the classification of antibiotics, their transformation and fate in biological treatment units can be summarized as the following:

4.2.2.1 β-Lactams

Despite that β-lactams are the most consumed antibiotics for humans and animals, they have not been detected frequently in WWTPs [14, 57], thus there was not much-published data about the fate and transformation of β-lactams in WWTPs. Junker et al. [57] studied the fate of some ^{14}C-labeled antibiotics (benzylpenicillin and ceftriaxone) in the activated sludge process. The results showed that ceftriaxone was not totally mineralized, whereas only about 25% of benzylpenicillin was mineralized. The same results were obtained using biodegradability tests (closed bottle tests method; CBT) at much higher concentrations of β-lactams antibiotics since ceftriaxone was kept unchanged whereas benzylpenicillin was biodegraded up to 27% [58].

The differences in biodegradability between β-lactams antibiotics may be due to the differences in their chemical structures because of diverse side chains [24, 59]. Andreozzi et al. [50] performed a standard batch experiment to study the fate of

amoxicillin in the activated sludge process. The results proved that the adsorption and biodegradation processes were responsible for the removal and transformation of amoxicillin.

4.2.2.2 Sulfonamides

Most researchers who studied sulfonamides in WWTPs focused on sulfamethoxazole and N4-acetylsulfamethoxazole (a metabolite of sulfamethoxazole). Batt et al. [41] and Pérez et al. [60] found that sulfonamides were biodegraded to a certain degree (low removal efficiency) in the wastewater biological treatment stage. Sulfonamides were removed during the biological treatment process with an average removal efficiency of 25% [13, 38], as well as sulfamethoxazole showed poor removal efficiency of 20% [42, 61].

Also, some studies reported the resistance of sulfonamides to different treatment processes during wastewater treatment [11, 12, 62]. Nevertheless, some other researchers reported the relatively high removal efficiency of sulfamethoxazole like 55% [34], 56% [63], 66% [64], 67% [65], and 74% [63].

The significant variation in removal efficiencies of sulfonamides during the biological treatment process can be attributed to the following reasons: first, the transformation of some metabolites such as N4-acetylsulfamethoxazole to the parent molecule (sulfamethoxazole) in the influent. Second, the mentioned removal efficiencies depended on grab or composite samples (24 h), which cannot reflect the whole treatment process [12, 66].

Thus, to avoid the previous limitations, some researchers used well-controlled laboratory reactors for studying the fate of sulfonamides and their removal pathway during the activated sludge treatment process [8, 67]. The biodegradation process of three sulfonamides at low concentrations (20 μg/L) using activated sludge reactors was studied by Pérez et al. [60] and they reported that the biodegradation process was so efficient and was able to remove the three sulfonamides for 3 days. Less than 26% of the initial antibiotics' concentrations were present by the third day, whereas by the tenth day, the removal efficiency increased up to 93%. In addition, it was reported that some microorganisms can utilize sulfamethoxazole as a carbon and/or nitrogen source after 3 days lag phase [68]. Despite these studies proving that sulfonamides can be biodegraded, the biodegradation process takes a long time than the usual hydraulic retention time of the biological treatment process at WWTPs.

4.2.2.3 Quinolones

Batt et al. [41] and Xu et al. [12] reported that adsorption followed by biodegradation are the main pathways for the removal of quinolones during biological treatment stages at WWTPs. The removal efficiencies for norfloxacin, ofloxacin, and ciprofloxacin were 87–100%, 75–77%, and 85%, respectively [69, 70]. The adsorption mechanism of quinolones by sludge depends on electrostatic interactions between particles rather than hydrophobic forces [21, 42].

4.2.2.4 Tetracyclines

In the biological treatment process at WWTPs, adsorption is considered the main mechanism for the removal of tetracyclines [41, 71, 72]. Tetracycline (10 μg/L) was

removed (up to >95%) rapidly through an adsorption mechanism during 6 h inside activated sludge units. Also, two lab-scale Sequencing Batch Reactors (SBR) were utilized to stimulate the activated sludge process (biological treatment), in these SBRs, the effect of SRT (Sludge Retention Time) and HRT (Hydraulic Retention Time) on transformation and fate of tetracycline were studied (66). The results showed that the removal efficiency of tetracycline in phase 1 (SRT = 10 days; HRT = 24 h) was 86.4 ± 8.7% and phase 2 (SRT = 10 days; HRT = 7.4 h) was 85.1 ± 5.4%, while in phase 3 (SRT = 3 days; HRT = 7.4 h) was 78.4 ± 7.1%. In phase 3, it was clear that the removal efficiency of tetracycline decreased by a reduction in SRT, which indicated that more tetracycline can be adsorbed by old sludge. In addition to that, it was reported that ferrous chloride could enhance the removal of tetracycline through precipitation due to the strong complexation between tetracyclines and ferrous ions [41].

4.2.2.5 Macrolides

All previous studies have indicated that all macrolides were not significantly eliminated, even at low concentrations, during the biological treatment process at WWTPs [10, 67].

4.2.2.6 Trimethoprim

Many studies indicated that trimethoprim was not adsorbed during the Activated Sludge (AS) process [41, 60]. The studies also proved that trimethoprim cannot easily biodegrade during AS with a short sludge retention time [11, 57]. However, Pérez et al. [60] reported that trimethoprim was completely degraded by nitrifying activated sludge with long SRT within 3 days. The nitrifying bacteria present in the nitrifying activated sludge are responsible for trimethoprim degradation since it was noticed that when the activity of nitrifying bacteria is inhibited, the elimination efficiency of trimethoprim decreased from 70 to 25% [42, 73].

4.2.3 Digestion tank

A little number of studies were carried out to study the transformation and fate of antibiotics in digestion tanks because the digestion process is not applied at most of the WWTPs all over the world. Zhang and Bing [5] conducted a two-stage anaerobic sludge digester (SRT = 30 days) to study the fate of fluoroquinolones (norfloxacin and ciprofloxacin) and they reported the stability of these fluoroquinolones. The same results were reported by Lindberg et al. [66] that ciprofloxacin and norfloxacin showed no significant removal under mesophilic sludge digesters (38°C). In contrast, Du et al. [14] utilized anaerobic mesophilic sludge digesters to study the stability of trimethoprim and sulfamethoxazole and they reported the instability of trimethoprim and sulfamethoxazole.

This may be due to that both trimethoprim and sulfamethoxazole have no significant amounts in digested sludge while their concentrations are high in activated sludge. Gartiser et al. [74] studied the biodegradation of nine antibiotics under an anaerobic digestion process (35 ± 1°C) and they reported that the biodegradation process was inefficient for all nine antibiotics except in the case of benzylpenicillin which was biodegraded after 40 days lag phase.

4.3 Antibiotics transformation and fate in advanced treatment processes

4.3.1 Filtration

To improve the quality of treated effluent, some wastewater treatment plants apply advanced treatment units such as Membrane Filtration (MF) or Sand Filtration (SF). During sand filtration, the removal efficiencies of trimethoprim and clarithromycin were 60 and 15%, respectively [8, 34]. Nakada et al. [75] also reported the same results for trimethoprim since the removal efficiency was 55.2% in the sand filtration process. However, sulfamethoxazole and sulphapyridine showed lower removal efficiencies of 26.9 and 14.6%, respectively after the sand filtration process.

Moreover, clarithromycin, azithromycin, roxithromycin, and erythromycin-H_2O showed no removal at all during the sand filtration process which may be due to the presence of highly diverse and effective biofilm on the SF particles [75]. Watkinson et al. [38] reported that about 43 and 94% of total antibiotics were removed by the microfiltration process and reverse osmosis, respectively via eliminating the particles that adsorbed antibiotics on them. Also, the Nanofiltration (NF) process increased the removal efficiencies of antibiotics up to more than 95% for antibiotics such as tetracyclines [42, 76].

4.3.2 Disinfection

Table 9 summarizes the transformation and fate of eight classes of antibiotics after disinfection units at WWTPs. The disinfectants reacted fast with the antibiotics and

Antibiotic	Disinfectant	pH	Performance	
			Contact time (min)	Removal efficiency (%)
β-lactams				
Penicillin G	Ozone	7.7	1	–70
Cefalexin	Ozone	7.7	1	>99
Sulfonamides				
Sulfamethoxazole	Free available chlorine	7.3	1	−95
Sulphapyridine	Ozone	7.95	27	93.9
Fluoroquinolones				
Ciprofloxacin	Free available chlorine	7.7	1	>99
Enrofloxacin	Ozone	7.7	1	>99
Macrolides				
Azithromycin	Ozone	7.95	27	92.6
Erythromycin-H_2O	Ozone	7.95	27	88.7
Clarithromycin	Ozone	7.2	18	>76
Tylosin	Ozone	7.7	1	>99
Roxithromycin	Ozone	7–7.5	8-40-4	>90–99
Tetracyclines				
Tetracycline	Ozone	7.7	1	>99

Antibiotic	Disinfectant	pH	Performance	
			Contact time (min)	Removal efficiency (%)
Amikacin	Ozone	7.7	1	−25
Vancomycin	Ozone	7.7	1	>99
Others				
Trimethoprim	Free available chlorine.	8.1	10	−100
	Ozone	7.7	1	>99
Lincomycin	Ozone	5.5	2	100

Table 9.
Transformation and fate results of antibiotics in the disinfection process.

the removal occurred after 1–27 min. In most studies, ozonation was applied for disinfection purposes. The ozonation oxidized antibiotics either directly by ozone (O_3) or by hydroxyl radicals (•OH). Hydroxyl radicals are generated due to the decay of ozone. Each ozone and hydroxyl radicals have different oxidation mechanisms since O_3 is selective and usually attacks the special functional groups (such as the aromatic structure or a C═C double bond), whereas hydroxyl radicals are non-selective and react with many types of moieties [75].

In addition, Cha et al. [77] studied the ozonation of 14 antibiotics and found that only 4 antibiotics including cephalexin, penicillin G, N(4)-acetylsulfamethoxazole, and amikacin were oxidized by hydroxyl radicals and the other 10 antibiotics reacted mainly with O_3. Moreover, by using other disinfectants such as hypochlorous acid (HOCl), some antibiotics such as trimethoprim showed no degradation, while sulfamethoxazole was degraded after a reaction with HOCl [78]. Liu et al. [10] reported that there was a significant variation in reaction rates of combined chlorine and free available chlorine with antibiotics in wastewater.

5. Conclusion

This review provides insight into the occurrence and fate of antibiotics in domestic wastewater treatment plants. Data was collected about the occurrence of the most widely six groups of antibiotics used for human cure including β-lactams, sulfonamides, quinolones, tetracyclines, macrolides, and others in wastewater and sludge samples of wastewater treatment plants. All previously mentioned groups of antibiotics were detected in wastewater and sludge samples with varied concentrations during the different treatment stages. It was clear that most of the wastewater treatment plants do not have the ability to fully remove these antibiotics. The main removal mechanisms of these antibiotics were biodegradation, adsorption, membrane filtration, and disinfection.

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