

Review

Impact of Antibiotic Pollution on the Bacterial Population within Surface Water with Special Focus on Mountain Rivers

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Abstract: Environmental aquatic pollution with antibiotics is a global challenge that affects even pristine mountain environments. Monitoring the concentration of antibiotics in water is critical to water resource management. In this review, we present the sources and degradation routes of antibiotics polluting surface waters, with particular focus on mountain environments and pristine areas. This pollution is strongly related to anthropopressure resulting from intensive tourism. An important aspect of the threat to the environment is water containing antibiotics at sub-inhibitory concentrations, which affects bacterial populations. Antibiotics are ecological factors driving microbial evolution by changing the bacterial community composition, inhibiting or promoting their ecological functions, and enriching and maintaining drug resistance. We paid attention to the stability of antibiotics and their half-lives in water related to biotic and abiotic degradation, which results from the structures of molecules and environmental conditions. Wastewater treatment combined with advanced treatment techniques significantly increase the efficiency of antibiotic removal from wastewater. Modern methods of wastewater treatment are crucial in reducing the supply of antibiotics to aquatic environments and enhancing the possibility of economic and safe reuse of wastewater for technical purposes. We provide a perspective on current research investigating antibiotic emergence in mountain areas and identify knowledge gaps in this field.



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Keywords: antibiotic-resistant bacteria (ARB); antibiotic resistance genes (ARG); bacterial populations; half-life; mountain rivers; tourism; water quality; wastewater treatment techniques

1. Introduction

Antibiotics are bactericidal and bacteriostatic agents used to treat bacterial infections, thereby providing a solution in the treatment of many diseases. Antimicrobial substances are also used for non-medical purposes, such as livestock, poultry, and fish growth stimulation. Remarkably, more antibiotics are used in the US for animal growth promotion than in human medicine [1]. Approximately 24.6 million pounds of antibiotics are used each year in livestock farming [2]. Animal husbandry utilizes more antibiotics than human therapeutic applications of these drugs. Importantly, the increased consumption of antibiotics has led to the exposure of aquatic ecosystems to contamination with these substances. This is because most antibiotics are only partially metabolized by the target organism; therefore, their residues (30–90% of ingested antibiotic doses) are excreted in urine and feces to reach wastewater treatment plants (WWTPs) [3]. Wastewater contaminated with antibiotics undergoes treatment in treatment plants but complete removal of these compounds is impossible in conventional systems [4]. WWTPs are designed to reduce the pollutant load in the majority of urban and rural wastewater, but they are not effective in reducing the loads of antibiotics and antibiotic resistance genes (ARGs) [5]. Furthermore, WWTP effluents are discharged into surface water and the sludge can be used as manure fertilizer. Antibiotics are a problem around the world due to their frequent

widespread use in large amounts, and they often become an inappropriate therapeutic pathway. Antibiotics are regarded as “pseudo-persistent” contaminants because of their continuous introduction into ecosystems—their entry rate into the environment is higher than their rate of elimination [4].

The presence of antibiotic residues in surface waters has been reported to influence the compositions and functions of microbial communities. Antibiotics dissolved in surface waters reach sub-inhibitory concentrations that affect the microbial ecology by increasing the mutation rate, causing horizontal drug resistance gene transfer, and driving the selection of antibiotic resistant bacteria [6]. Antibiotic use in human medicine, agriculture, aquaculture, and veterinary medicine puts a huge selective pressure on microbial communities [6]. If the selective pressure is high enough, acquisition of resistance becomes necessary for bacterial survival. Long-term exposure to sub-inhibitory concentrations of antibiotics in the aquatic environment could be the main factor responsible for the generation of drug resistance and transference of drug resistance genes [7,8].

Antimicrobial resistance determinants are commonly found in wastewater [5], thus posing a threat to the environment resulting from the possibility of drug resistance spread. Overuse and misuse of antibiotics contribute to the evolution of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). The pollution of rivers by ARB and ARGs increases the risk of antibiotic resistance transmission from the environmental resistome to humans [9] and also affects the ecological balance of the aquatic environment [10]. Antibiotic resistance genes are recognized as emerging micropollutants with environmental persistence. Antibiotic resistance genes are present in both extracellular (eARGs) and intracellular (iARGs) forms in the environment [11]. However, eARGs play a crucial role in the spread of antibiotic resistance in the environment thanks to their ability of being absorbed on soil and sediment particles, thus persisting longer in water environments [12]. Horizontal gene transfer (HGT) enables the transport of ARGs by mobile genetic elements between bacterial cells. It allows dissemination of ARGs from commensal and environmental bacterial species to pathogenic species [13]. Water is a major pathway of dissemination of bacteria and ARGs between different environmental compartments. Human exposure could occur through contaminated recreational areas as well drinking and recreational water [14]. The problem of surface water contamination is very common [15–18], even in river waters in natural reserves that are expected to be clean [19].

The next-generation sequencing (NGS) technique has recently become the most frequently applied method for examining microbial community structure in water environments [20–23]. The total bacterial community plays an important role in aquatic ecosystems [23]. The NGS method allows researchers to determine the presence of all species of bacteria present in the studied environment, even non-cultivated ones. Therefore, the NGS method could become a useful tool for water quality monitoring purposes, including the development of bioindicators for sewage pollution and microbial source tracking [21].

Contamination of the aquatic environment with antibiotics is of interest to scientists, as evidenced by numerous scientific publications presenting the results of research investigating the concentrations of antibiotics in waters, their impact on the environment, and the risks resulting from this phenomenon. It should be noted that mountain regions are the sources of river systems that provide fresh water to more than half of the human population [24]. Human wellbeing depends on mountain resources because they provide clean water and harbor rich biodiversity. Water from mountain rivers is exploited for many purposes, including drinking water sources, irrigation, recreation, and snowmaking on ski slopes, which is common in these areas to ensure the operation of ski stations. In many countries, due to highly developed tourist infrastructures and accommodation facilities, mountain areas are characterized by a large amount of generated sewage. This is due to the growing number of residents in peak tourist seasons, which results in significant overload and decreased efficiency of local treatment plants [25]. The above described key function of mountain river systems may be disturbed by the impact of human pressure in this environment. Surface water contamination in mountain areas, particularly protected ones,

and most importantly the contamination of mountain river waters, is one of the greatest issues of water management. The reason for this is because mountain river waters are particularly sensitive to both natural and anthropogenic changes due to their cleanliness.

For the above mentioned reasons, the aim of this review was to analyze the sources and stability of antibiotics in water, including the rivers of protected mountain areas. The review presents the anthropogenic pressure in mountain regions, which is mainly related to tourism, that contributes to the contamination of water systems with antibiotics. This review also presents the impact of this pollution on the composition of microbial populations and the development of drug resistance. Most importantly, this paper summarizes and compiles the most up-to-date research concerning issues related to the antibiotic contamination of surface water, with particular focus on mountain and pristine areas, and identifies knowledge gaps and ways to fill them within the discussed scope.

In this regard, we present a systematic review of the latest literature on antibiotic contamination of the aquatic environment, with special emphasis on mountain areas. The search strategy to prepare this research review included browsing through the following databases: Scopus, Science Direct, PubMed, Tylor, and Google Scholar. Descriptors such as antibiotics, antibiotic agents, drug-resistant bacteria, bacterial community structure, mountain water pollution, alpine rivers, mountain springs, pristine environment, tourism traffic, and their combinations were used to search for information on mountain water pollution with antibiotics. All science databases were searched for papers published in the English language and were not restricted to any specific region in order to find studies conducted worldwide. The period of time that was set for searching articles covered the years 2003–2023. We also used two articles published in 1985 and 1999 that dealt with the level of excretion of antibiotics by humans and animals. After the application of the above search strategy, very few studies (7 articles) were obtained that examined the occurrence of antibiotics precisely in mountain rivers. This indicates a substantial knowledge gap that needs to be filled or is being investigated with respect to studies focused on the mountain environment. A significant number of studies present the results on surface water pollution in urban areas, often resulting from the presence of sewage treatment plants and the resulting consequences for microorganism populations and humans. Considering the above, a literature review was created using the available information on the contamination of mountain waters with antibiotics in relation to the general contamination of surface waters by these substances.

2. Sources of Antibiotics in Mountain Rivers

The quality of water in mountain regions is shaped by many natural and anthropogenic factors. In the most pristine regions, including national parks and natural valuable protected areas, the natural factors include variable weather conditions, surface runoff, soil leaching, and snowmelt water [26,27]. Along the course of rivers, water pollution increases, which is strongly related to the influence of anthropopressure-related factors, including illegal discharge of sewage from households (human and animals feces), surface runoff carrying natural fertilizers from agricultural fields, and wastewater inflow from WWTPs increased by tourist traffic-related sewage inflow [26,28].

In many cases, anthropogenic pressure in mountain regions is related to tourist traffic contributing to increased wastewater inflow, thus contributing to the presence of antibiotics and bacterial contaminants in mountain rivers [25]. Constantly developing winter tourism can pollute the environment and affect the ecology of microorganisms in mountain aquatic ecosystems in various ways. Mountain hiking is one of the causes of the pollution of rivers with antimicrobial agents. Mountain areas attract tourists due to their unspoiled nature, hiking trails for mountain trekking, and areas for active recreation. One of the reasons for the growing number of tourists is the constant development of winter sports centers with snow-covered ski slopes, which also offer activities outside the winter season, such as downhill skiing, as well as facilities offering thermal pools with geothermal water (thermal spas). Excluding the Alpine countries and the United States, Poland is at the

forefront of the development of ski infrastructure [29]. In the winter season of 2014/2015, one of the several ski stations located in the Białka river valley (Podhale, Southern Poland) was visited by approximately 318,000–342,000 people [30]. The same ski resort offered 6500 beds in 2012, while this number increased to 13,200 beds in 2020. Moreover, the transport capacity of ski lifts in 2020 was 32,975 people per hour. At the same time, the number of inhabitants in this mountain village was 2249 people as of December 2019 [31]. The presented numbers of tourists compared to the number of inhabitants for only one mountain village in Poland show a possible negative impact of tourism on the pollution of mountain rivers. Ski resorts exert a significant pressure on adjacent natural areas, which is often more important than the impact of general tourist activity located further from the ski resorts. Most importantly, the greatest negative pressure is exerted on water quality [32]. Samples of snow from the Sudety Mountains in Poland, collected in places with different levels of anthropogenic pressure, indicated that the presence of humans could affect the composition of the microbial resistome in snow [33]. In Central Europe, the most attractive countries in terms of winter sports and mountain hiking are Austria, Slovakia, the Czech Republic, and Poland [34]. Ski resort owners in all of these countries must comply with the legal aspects of environmental protection. Many ski resorts and mountain hiking areas in these countries are located on territories included in Natura 2000, the international network of protected areas covering Europe's most valuable and threatened habitats [35,36]. There is a conflict between the constant development of mountain tourism and keeping the environment and rivers unchanged [37]. The Tatra Mountains, located on the border of Poland and Slovakia, are among the most frequently visited regions in these countries. The Tatra National Park (TNP) offers 275 km of hiking trails, 6 bicycle routes, and 8 tourist shelters with the availability of gastronomy and accommodation. The number of tourists visiting the TNP is growing year by year, from 2,926,012 tourists in 2014 to 4,600,025 tourists in 2021. On average, the TNP is visited by 3 million tourists each year [38]. Such great interest in mountain tourism is a burden on the environment. Mountain shelters located in the TNP are not connected to the sewerage system. They only have biological treatment plants or septic tanks, from which waste must be transported to treatment plants outside the TNP. According to an interview with TNP authorities (February 2020) [39], approximately 30 cubic meters of wastewater are generated per day in the mountain shelter on Morskie Oko Lake (one of the most recognizable of the Tatra lakes), which is visited by approximately 10,000 people per day in peak season. Such large amounts of wastewater produced in the national park have a significant impact on the pollution of mountain streams, with antibiotics and drug resistance determinants flowing down to the rivers from which water is drawn for a number of purposes, including snowmaking or irrigation of green areas. Lenart-Boroń et al. [40] examined the occurrence of antibiotics in the groundwater of pristine areas in the Tatra National Park and in waters of one of the mountain rivers. None of the tested antibiotics were detected in one of the groundwater sampling sites in the TNP area (located at 1600 m a.s.l.), but the second groundwater sampling site, still located in the TNP but much closer to households and located below mountain shelters, was contaminated with antibiotics at the following mean concentrations: erythromycin (0.89 ng/L), ofloxacin (0.27 ng/L), clindamycin (0.36 ng/L), vancomycin (2.99 ng/L), trimethoprim (0.29 ng/L), and sulfamethoxazole (0.20 ng/L). The total concentration of antibiotics at one groundwater site was 27.92 ng/L. River water in the TNP pristine area contained a total of 34.02 ng/L of antibiotics, including erythromycin, oxytetracycline, clindamycin, and vancomycin. The above-described considerable intensity of mountain tourism influences changes in the natural environment in a number of ways. Among them, increased water consumption and the production of wastewater are the main problems [24,28,32,41,42]. Antibiotics are continuously discharged into wastewater after their metabolism in target organisms via excretion in urine and feces from human and animal bodies. Importantly, most antimicrobials are not completely metabolized (the range of metabolism is 10–90%, Table 1) [43]; therefore, both the parent compounds and degradation products reach wastewater treatment plants. In addition, some metabolites,

such as acetic conjugates of sulphonamides, can revert back to their parent compounds during manure storage [44].

Table 1. Levels of antibiotics excreted in unchanged form after administration to humans (h) or animals (a).

Antibiotic	Class	Excretion Level	Reference
Levofloxacin	Fluoroquinolones	85% (h)	[45]
Sulfamethoxazole	Sulfonamides	12% (h)	[46]
Trimethoprim	Nitroimidazoles	60% (h)	[46]
Metronidazole	Nitroimidazoles	60–80% (h)	[47]
Erythromycin	Macrolides	5% (h)	[48]
Ofloxacin	Fluoroquinolones	80% (h)	[48]
Tetracycline	Tetracyclines	80% (h)	[49]
Tylosin	Macrolides	40% (a)	[50]
Oxytetracycline	Tetracyclines	21% (a)	[51]
Chlortetracycline	Tetracyclines	17–75% (a)	[51]

The metabolism rate of these compounds varies for humans and animals depending on the antibiotic class. These pharmaceuticals may reach aquatic environments by effluents from WWTPs to rivers and groundwater, as well as leachate from unsealed sewage systems and manure and/or sewage storage tanks [52]. During the wastewater treatment process, bacteria are continuously mixed with sub-inhibitory concentrations of antibiotics, which creates suitable conditions for the development of drug resistance, and then released with antibiotic residues into water environments [53]. The removal efficiency of antibiotics and antibiotic resistance determinants from wastewater by conventional WWTPs is insufficient. WWTPs are not specifically designed to completely reduce levels of antibiotics and ARGs. Removal of different antibiotics occurs in different steps of the wastewater treatment process, and the effectiveness of their removal varies among antibiotics [5]. Removal efficiency depends on the physical and chemical properties of antibiotics and on the treatment process conditions. Blair et al. (2015) [54] evaluated the maximum concentrations of pharmaceuticals within conventional activated sludge treatment processes. The results showed the presence of nine antibiotics: ampicillin (160 ng L^{-1}), ciprofloxacin (2200 ng L^{-1}), enrofloxacin (34 ng L^{-1}), norfloxacin (140 ng L^{-1}), ofloxacin (2100 ng L^{-1}), penicillin G (30 ng L^{-1}), penicillin V (86 ng L^{-1}), sulfamethoxazole (7400 ng L^{-1}), and trimethoprim (570 ng L^{-1}). The removal efficiency for all antibiotics except ampicillin was negative in this research. WWTPs have been recognized as hotspots (as the main sources of antibiotics) for the release of antibiotics into the aquatic environment. In order to remove organic pollution from wastewater, activated sludge systems are sometimes combined with chemical additions [55]. However, previous studies have concluded that conventional secondary treatment processes are unable to completely remove antibiotics from wastewater [56,57]. Advanced wastewater treatment techniques, such as membrane processes, activated carbon adsorption, and UV radiation, may increase the percentage of antibiotic removal from wastewater and are presented later in this review. WWTPs have emerged as significant sources of antibiotics to mountain aquatic environments due to the contamination of WWTP influent by antibiotics from human and animal excreta, as well as improper disposal of antibiotics and agriculture runoff. The risk of contamination of surface water with antibiotics, drug resistance genes, and drug-resistant bacteria also results from the utilization of sewage sludge in agriculture as an organic fertilizer [58]. Sewage sludge, contaminated with antibiotics and drug resistance determinants, flows from the soil into rivers along with surface runoff. The most common antibiotics found in sewage sludge are fluoroquinolones, sulfonamides, and tetracyclines, the concentrations of which were measured at $\mu\text{g kg}^{-1}$ [59–62].

Another significant source of antimicrobials in rivers, including mountain rivers, is their use in animal husbandry for therapeutic and preventive purposes. Mountain

agriculture is dominated by livestock production based on grazing. Veterinary antibiotic usage is related to the treatment of infective diseases in animals. The use of antimicrobial agents in animal husbandry ensures the welfare and health of the animals. However, the use of antibiotics is extended to the whole livestock flock in order to limit pathogen spread, thus uninfected animals also take doses of antibiotics [63]. This is referred to as metaphylaxis—short-term antibiotic treatment of animal groups without disease symptoms that had contact with infected animals [64]. This action involves observation of a livestock flock and administration of high doses of antibiotics before clinical symptoms occur in order to counteract the effects of infection. In contrast, antibiotics can also be used for disease prevention (prophylaxis). This includes antibiotic administration in water and food for farm animals in low doses for longer periods of time. During this period, the risk of infection still exists [65]. Metaphylaxis and prophylaxis are common practices in livestock and poultry production to prevent whole livestock mortality and minimize losses, but they have boosted antibiotic consumption. From an epidemiological point of view, the preventive administration of antibiotics increases the risk of drug-resistant bacteria development in the livestock herd and significantly influences the contamination of the environment with antibiotics and drug resistance determinants. In addition, selection for antibiotic-resistant strains can be widespread in the environment via animal feces, thereby enhancing environmental drug resistance [66]. Residues of antibiotics and ARB are usually found in livestock and poultry manure and in waste from livestock companies, resulting in persistent environmental pollution [67]. Animal manure studies have proven the presence of various classes of antibiotics excreted in feces, for instance: enrofloxacin in broiler chicken feces (74% of orally applied enrofloxacin was excreted as the parent compound) [68], oxytetracyclines present in dairy cow feces (20% of injected oxytetracycline was detected in manure samples) [69], and sulfonamides in pig excreta (excretions of four sulfonamides reached 36–87%) [70]. Stored animal manure often reaches soil and surface water with runoff water after rain or due to leaks in manure tanks. Livestock manure is also used as a fertilizer to enrich the soil before growing crops. Mountain areas, in addition to their environmental and cultural functions, also have an agricultural function as they have abundant arable fields, meadows, and pastures. In sustainable and organic farming, the use of manure as a source of organic matter to improve soil quality is a common practice. However, manure is also a source of antibiotic residues, which can adsorb on soil particles, enter plant tissues, and end up in the food chain. There is a risk of enhanced antimicrobial resistance as a result of consumption of vegetables grown on manure [71,72]. Manure widely applied to agricultural lands as fertilizer has enriched the abundance of some ARGs (*ermA*, *ermB*, *blaOXA-1*, *qnrS*, and *oqxA*) in agricultural soil [73]. Antibiotic residues and drug resistance determinants in soil fertilized with manure enter rivers with surface runoff, thus polluting the aquatic environment. Active forms of antibiotics occurring in manure can act as a selective pressure and contribute to dissemination of antimicrobial resistance. Livestock animals are a constant link in the spread of ARGs and antibiotics in the aquatic environment because they are continuously exposed to large amounts of antibiotics. Livestock farming can be one of the main sources of antibiotics in rivers due to the excretion of incompletely metabolized antibiotics in animal feces and their further dissemination into the environment [67].

Mountain rivers provide water for the production of artificial snow to ensure snow cover on ski slopes in the winter season and for irrigation of green areas in the summer season. Mountain river water is also used by households to irrigate their crops. The use of water contaminated with antibiotics, drug resistance genes, or antibiotic-resistant bacteria results in further transmission of these micropollutants into the environment, thereby increasing the risk of spreading drug resistance and endangering public health (Figure 1).

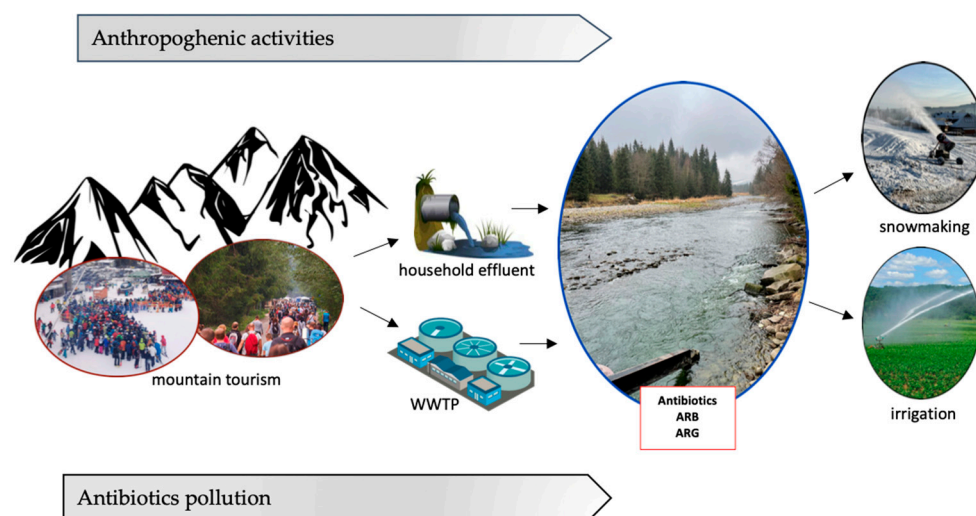


Figure 1. Dissemination routes of antibiotics and drug resistance determinants in the mountain environment.

Research on the microbiological pollution of the two largest rivers of Podhale (Tatra Mountains, Southern Poland), which are the source of water for snow production on ski slopes, conducted over two ‘high ski seasons’ demonstrated the bacterial pollution of both the river and fresh artificial snow produced from these waters. Bacterial indicators of fecal contamination (coliforms, *E. coli*, and *E. faecalis*) were observed in two out of seven samples of artificial snow produced from polluted water. Furthermore, *E. coli* strains presented the ESBL (extended-spectrum beta-lactamases) resistance mechanism and contained the *bla*TEM gene in their genome, as confirmed by the PCR method [74]. These observations indicated that the antibiotic-resistant bacterial strains were able to survive the production of artificial snow and that users of the ski slopes were exposed to direct contact with drug-resistant bacteria. Sanchez-Cid et al. [33] evaluated the bacterial community composition and microbial resistome in natural snow samples from mountain tourist attractions and forest areas in the Sudety Mountains, Poland. ARG reads from metagenomic sequencing of snow-derived DNA using the MiSeq System (Illumina, San Diego, CA, USA) were grouped by antibiotic class and their average abundance was compared between the studied sites. The sites with larger surrounding forest areas showed a higher ARG abundance. Several genes showed higher abundance in samples from paths with the highest human activity. These genes determined the resistance to: aminoglycosides (*aadA17* gene), tetracycline (*tetX* gene), rifamycin (*rphB* gene), fosfomycin (*fosA5* gene), beta-lactams (*rm3* and *LRA-13* genes), and the multidrug resistance gene *meI* [33]. The results suggested that anthropogenic activities could have a direct impact on the composition of the antibiotic resistome in snow. Furthermore, Segawa et al. [75] observed the prevalence of antibiotic resistance genes in glacier environments (snow and ice samples). ARGs, of both clinical (*aac* (3), *bla*IMP) and agricultural (*strA* and *tetW*) origin, were detected. These researchers indicated that ARGs in such pristine environments can be transferred by airborne bacteria and migratory birds. Research shows that determinants of drug resistance in the form of ARGs and antibiotics can be stable in the water environment and penetrate into other environmental compartments, such as snow or ice. In Yang and Carlson’s [76] study of river antibiotic contamination in Colorado, the only site in which no antibiotics were detected was a pristine mountain site upstream of urban or agricultural areas. Conversely, all five monitored tetracyclines were detected at a site that had undergone both urban and agricultural impacts at concentrations ranging from 0.08 to 0.30 mg/L.

Another essential route of further transmission of antibiotics in the mountain environment is the irrigation of fields with antibiotic-contaminated water [77]. Irrigating crops and green areas with antibiotic-contaminated water leads to crop contamination and dis-

semination of drug resistance genes [78]. Antibiotics from irrigation water can accumulate in the edible parts of plants or grasses on which livestock feed. Plants irrigated with antibiotic-contaminated water increase the threat of adaptive resistance selection of the gut microbiome. The amounts of antibiotics found in the environment are considered as trace contaminants, nevertheless, they have a very significant impact on the environment [25]. Although the concentrations of antibiotic residues in water environments range from ng/L to $\mu\text{g/L}$ [79], the continuous discharge and persistence of these contaminants at sub-inhibitory concentrations may cause changes in bacterial communities and stimulate the development of drug resistance. The transference of drug resistance genes from environmental bacterial strains to human pathogens is a major threat to public health. Water-polluting antibiotics cause the development of antimicrobial resistance among microorganisms, hence their presence in the environment is of critical importance to public health.

3. Stability of Antimicrobial Agents in Water Environments

Antibiotic degradation rates are important for predicting their environmental exposure and impact on bacterial populations. Antibiotics dissolved in water undergo physicochemical modifications caused by biotic and abiotic factors that affect their structural stability. The following processes affect the stability of antibiotics in surface water: hydrolysis, photolysis, sorption, and biological degradation [80–84]. The occurrence of these processes depends on environmental conditions such as sunlight, water temperature, the abundance of microorganisms, water chemical composition, sediment properties, and organic matter content. Predicting the degradation pathways of antibiotics is essential for assessing their fate in the environment. The natural degradation pathways of antibiotics in water environments are presented in Figure 2.

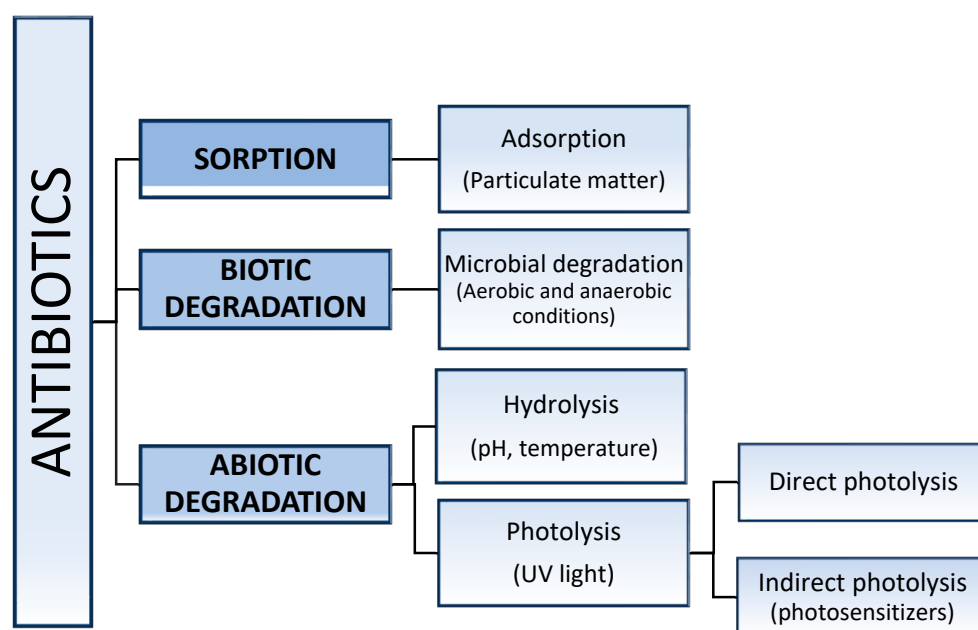


Figure 2. Natural degradation pathways of antibiotics in water environments.

An important factor affecting environmental contamination with parent antibiotics is their duration in water while they still have bactericidal properties before they decompose into transformation products. The half-life of an antibiotic in water is an important factor in determining its concentration in an aquatic sample. The literature data showing half-lives of parent antibiotic compounds in aquatic environments are summarized in Table 2.

Table 2. Half-lives ($T_{1/2}$) in days (d) or hours (h) of antibiotics in different water samples under aerobic conditions and daylight.

Chemical Groups	Compound	Sample Type	Temp. [°C]	T _{1/2}	Reference
Cephalosporin	Cefradine 1st	lake water	25 ± 3	6.3 d	[83]
	Cefuroxime 2nd			3.1 d	
	Ceftriaxone 3rd			18.7 d	
	Cefepime 4th			2.7 d	
(Amino)penicillin	Amoxicillin	ultrapure water	19 ± 0.5	3.32 ± 0.61 h	[85]
	Ampicillin			3.89 ± 0.43 h	
	Penicillin V			4.37 ± 0.22 h	
	Piperacillin			6.99 ± 0.45 h	
Tetracycline	Tetracycline	river water	25 ± 1	4.15 d	[86]
	Oxytetracycline	river water		1.82 d	
	Chlortetracycline	surface water		3.35 h	
Sulfonamide	Sulfamethoxazole	surface water	25 ± 1	14.22 h	[87]
		STP effluents	Winter	2.4 d	[88]
		river water	25 ± 1	17.8 d	[86]
	Sulfamethazine	surface water	25 ± 1	1.3 d	[87]
		river water		17.3 d	[86]
	Fluoroquinolones	Enrofloxacin	surface water	25 ± 1	3.34–6.75 d
river water			8.78 d		[86]
Ciprofloxacin		deionized water	19± 1	0.33 h	[89]
		kaolinite suspension	19± 1	1.2 h	[89]
		river water	25 ± 1	5.33 d	[86]
Ofloxacin		STP effluents	winter	10.6 d	[88]
		river water	25 ± 1	11.1 d	[86]
Norfloxacin		river water	25 ± 1	5.64 d	[86]
Macrolides	Erythromycin	sea water	18± 2	11.11 d	[90]
		river water	25 ± 1	4.22 d	[86]
	Roxithromycin	wastewater	4	2.9 d	[91]
		river water	25 ± 1	2.76 d	[86]
	Clarithromycin	wastewater	4	2.9 d	[91]
Azithromycin	4.8 d				

Photocatalytic degradation and hydrolysis are two of the main abiotic pathways of antibiotic degradation in aquatic environments [80–82]. The degradation of antibiotics in water depends on the pH value and temperature, which are the most important parameters affecting hydrolysis rates. These rates typically increase when the temperature increases. Additionally, aqueous compounds such as metals and organic matter can catalyze the hydrolysis reaction. Hydrolysis is the main degradation pathway in aquatic environments without abundant microbial populations, such as rivers and streams, but biodegradation pathways are predominant in wastewater where microbial populations are much more abundant than in surface waters [80]. Fang et al. [82] reported that the pH value had a significant effect on the elimination of enrofloxacin in aquaculture water. Among the tested pH values of 5, 7, and 9, the enrofloxacin removal rate was highest at pH 5 and 25°C . The half-life for enrofloxacin in water ranged from 3.34 to 6.75 days. In addition, the main degradation product of enrofloxacin is ciprofloxacin, an antibiotic from the fluoroquinolone class [90,92]. On the other hand, Mitchell et al. [80] determined that the hydrolysis of β -lactam antibiotics, ampicillin, cephalothin, and cefoxitin, was most effective at pH 9 and 25°C , while antibiotic hydrolysis was independent of pH within pH range 4 to 8. The half-lives for cephalothin, cefoxitin, and ampicillin under these conditions were 1.4, 6.6, and 6.7 days, respectively. The authors also established that the

hydrolysis rate constants were approximately 20 times higher at 50 °C than at 25 °C. These results confirmed that hydrolysis rates were highly temperature dependent. A study on oxytetracycline hydrolysis showed 72.7% degradation at pH 6.91 after 6 days of incubation at 25 °C. On the other hand, oxytetracycline incubation at pH 3.09 at the same temperature resulted in the hydrolysis of 10.5% of oxytetracycline [81]. The presented research results showed that the rate of hydrolysis depends on the chemical structure within various classes of antibiotics, temperature, and pH value. Antibiotics have to be stable under the acidic conditions in patients' stomachs. Aquatic environmental conditions are around pH 7 at a temperature below 24 °C. Under such conditions, antibiotics can undergo hydrolysis. β -lactam antibiotics are subject to the hydrolytic cleavage of the β -lactam ring, especially under alkaline conditions [83]. Hydrolysis of the β -lactam ring in the antibiotic molecule causes the loss of bactericidal properties. Research results indicated that the predicted β -lactam antibiotic hydrolysis under ambient pH and temperature conditions and their degradation occurred within a few weeks in most surface waters [80] or even a few hours in ultrapure water [83]. Importantly, antibiotics are continuously released into water systems, which could result in their constant occurrence in the aquatic environment.

The second essential pathway for the degradation of antibiotics in water is photolysis. Degradation of antibiotics can occur through direct photolysis, which is caused by direct absorption of solar light, or indirect photolysis, which involves natural photosensitizers like nitrate and humic acid suspended in water. Under solar radiation, these constituents can generate excited compounds such as hydroxyl radicals and singlet oxygen [88,93]. In addition, organic matter dissolved in water is characterized by high mobility and can promote the solubility of organic pollutants in surface water [88]. Photolysis rates vary along with season, time of day, and water depth [87]. The results obtained by Wei et al. [87] in their study on the photo-transformation of tetracycline and sulfonamide antibiotics in surface water indicated that the decomposition of antibiotics could be dominated by direct photo transformation in summer. However, the half-lives of sulfonamide antibiotics could reach 1 month in the winter season. Sulfonamides are very sensitive to seasonal variations of UVB solar radiation. In winter, sulfonamide photo-transformation is replaced by sedimentation, adsorption, and biodegradation [87]. On the other hand, tetracycline antibiotics are unstable upon exposure to natural sunlight because the main absorption peak of these antibiotics (i.e., 365 nm) is well overlapped with the solar spectrum in the range of 290 to 420 nm [87]. Therefore, photolysis is the main pathway of tetracycline degradation in surface water. Direct photolytic degradation of antibiotics occurs mainly in the upper layer of surface water [85]. Aminopenicillins (amoxicillin, aminopenicillin) degrade faster under simulated sunlight conditions than penicillins (piperacillin, penicillin) [85,94]. Photolytic degradation of antibiotics in river water is much slower compared to that in ultrapure water due to organic matter absorbing the radiation and water turbidity [85]. Jiang et al. [83] determined that the half-lives of four cephalosporins (cefradine, cefuroxime, ceftriaxone, and cefepime) in surface water in the dark ranged from 2.7 to 18.7 days, while the half-lives of the cephalosporins decreased significantly to 2.2–5.0 days under exposure to simulated sunlight. The researchers concluded that direct photolysis was the primary process involved in the degradation of cephalosporins in the surface water of a lake. Photolytic degradation of antibiotics dissolved in water is particularly intense in summer when the intensity and exposure of sunlight is the strongest of the whole year. Therefore, due to low temperatures and less sunlight exposure, antibiotics are more stable in the water of mountain rivers in winter. Because of the extended stability of antibiotics in rivers, these compounds can enter other environmental compartments along with river water. One of such pathways is the production of artificial snow from river water in order to provide snow on ski slopes.

Antibiotics can also be retained in the aquatic environment by sorption with organic matter such as humic substances and organic carbon. Hydrogen bonds stabilize antibiotics on the surface of organic molecules [95]. Sorption of antimicrobial agents to the mineral components of the river sediment might protect these compounds against microbial degradation and thus prolong their half-lives in water [83]. For instance, oxolinic acid

(quinolone group antibiotic) is very stable in the aquatic environment (9 days in water and 48 to 300 days in sediment) and its long persistence in water is related to adsorption onto sediments [90]. Antibiotics with high adsorption coefficients may undergo repeated adsorption and desorption in the aquatic environment. An even greater ecological threat to the environment is the deposition of antibiotics in river water adsorbed on solid particles, such as micropollutants, which include microplastics occurring in the aquatic environment [96,97]. Antibiotics and microplastics are two classes of emerging pollutants with negative impact to the aquatic environment. Microplastics have different adsorption capacities for organic pollutants, including antibiotics, due to different surface characteristics, pore size distributions, and various degrees of crystallization [96]. Adsorption of antibiotics on microplastics could result in their long-range transport and increase their exposure to aquatic environments. The main sources of both antibiotics and microplastics in the aquatic environment are wastewater treatment plants, the effluents of which are point sources of these micropollutants in rivers [98]. Li et al.'s (2018) results indicated that polyamide particles have high adsorption for amoxicillin, tetracycline, and ciprofloxacin because of their highly developed pore structure [96]. Therefore, polyamide micropollutants can serve as carriers of antibiotics in the aquatic environment [96]. According to Wang et al. [97], increased water salinity could reduce the adsorption of antibiotics onto microplastics. Microplastics can concentrate more antibiotics and ARGs in fresh river water than in seawater. In addition, the amount of sulfamerazine, chloramphenicol, and tylosin adsorbed on polyethylene microplastics in river water was twice that in sea water [97]. Adsorption of antimicrobial compounds with slow-sinking organic particles enables the spread of these substances across long distances in the aquatic environment and protects these compounds against rapid degradation.

Antibiotics found in surface waters can be transformed by microorganisms through biological degradation pathways. It is possible to distinguish microorganisms capable of modifying (biotransformation), cleaving (biodegradation), or mineralizing (subsistence) antibiotics [84]. Some bacterial strains, called antibirotrophs, have the potential to use antibiotics as sources of carbon and energy and survive as antibiotic-resistant strains in environments with antibiotics as the sole carbon source [99]. Moreover, these strains often possess transmissibility of virulence and pathogenicity. Cha and Carlson [100], in their research on the biodegradation of veterinary antibiotics in lagoon waters, established that antibiotic biodegradation rates were faster under aerobic conditions. Additionally, biodegradation depended on ambient temperature, with elevated temperature (20 °C) increasing the rate of decomposition and lower temperature (4 °C) reducing the biodegradation rate. The lower efficiency of antibiotic biodegradation under anaerobic conditions was also observed by Jiang et al. [83] in their study on the biodegradation of cephalosporins in lake water. Additionally, these researchers established that biodegradation played a minimal role in cephalosporin decomposition, regardless of a diverse bacterial community. Yang et al. [101] revealed the roles of 24 bacterial genera in a microbial community involved in the aerobic and anaerobic degradation of amoxicillin, tetracyclines, and sulfonamides in wastewater sludge. *Pseudomonas* sp. strains present the greatest aerobic degradation capability. On the other hand, bacterial strains of *Bacillus* sp. and *Clostridium* sp. show the greatest anaerobic antibiotic-degradation capability. Microbial degradation shows promising prospects in the removal of sulfamethoxazole. Ammonia-oxidizing bacteria and sulfate-reducing bacteria present good removal capacity of sulfamethoxazole. However, low concentration of sulfamethoxazole could be insufficiently bioavailable for environmental bacterial strains [102]. Environmental conditions, such as temperature, pH, antibiotic concentration, and additional carbon sources, can affect the degradation of these micropollutants. Biodegradation pathways of antibiotics prevail in environments with abundant and diverse microbial populations e.g., in wastewater [80]. In river waters with lower abundances of microorganisms, the biodegradation of antibiotics occurs at a lower level. However, biodegradation may predominate when hydrolysis and photolysis do not show

much intensity, which depends on environmental conditions such as temperature, pH value, and the availability of sunlight.

4. Effect of Sub-Inhibitory Concentrations of Antibiotics on Bacterial Populations

Antibiotics entering aqueous environments as a result of anthropopressure could potentially affect the communities of microorganisms. They can be regarded as an ecological factor driving microbial evolution by changing the structures of microbial communities, inhibiting or promoting their ecological functions, and affecting drug resistance mechanisms [103]. The impact of antibiotics on the aquatic ecosystem is related to their concentrations, bioavailability, exposure time, and the addition of substrates, e.g., metals [103]. Antibiotic-induced changes in the ecological functions of the aquatic environment include the nitrogen transformation process, e.g., oxytetracycline inhibits the nitrification process in surface water [104]; however, in some cases, increased nitrification activity has been observed when bacteria are exposed to antibiotics [105]. Moreover, Fountoulakis et al. [106] reported that antibiotics could inhibit the methanogenesis process. In their research, sulfamethoxazole and ofloxacin mildly inhibited the anaerobic digestion process of methanogens. In other studies, Córdova-Kreylos and Scow [107], based on phospholipid fatty acid analysis, discovered that the broad-spectrum antibiotic ciprofloxacin favored the presence of sulfate-reducing bacteria and Gram-negative bacteria, such as *Desulfovibrio*, *Desulfobulbus*, and *Desulfobacter*, while reducing the number of Gram-positive bacteria in salt marsh sediment. In this research, ciprofloxacin was capable of modifying the bacterial community structure at concentrations as low as $20 \mu\text{g mL}^{-1}$ in anaerobic sediments. Importantly, despite the fact that the sorption of antibiotics on sediments is estimated at 80–90%, studies have shown that antibiotics at low bioavailability are still capable of modifying the microbial community.

Antibiotics polluting the natural environment do not reach the high therapeutic concentrations that inhibit the growth of bacteria ($\sim 1 \text{ mg mL}^{-1}$) [108]. However, they are widely distributed at low concentrations ($\text{ng} - \mu\text{g L}^{-1}$) [52,109] without reaching the minimum inhibitory concentration (MIC). The level of an antibiotic that is below the MIC concentration is referred to as sub-MIC (sub-minimum inhibitory concentration), or sub-inhibitory concentration in the literature. These levels are not considered as lethal concentrations, but they still affect individual cells of bacteria or their populations in various ways. The continuous increase in the prevalence of sub-inhibitory levels of antibiotics in the environment is a key aspect of the current problem of widespread drug resistance worldwide. Sub-inhibitory concentrations of antibiotics are increasingly found in many aquatic environments, such as sewage and sludge, rivers, lakes, and even drinking water and water in pristine environments [20,25,110–113]. Aquatic environmental concentrations of antibiotics reaching from ng/L to $\mu\text{g/L}$ are generally too low to inhibit bacterial activity, but environmentally relevant concentrations of antibiotics could enhance bacterial communication and transcriptional regulation. Sub-MIC concentrations of antibiotics found in the natural environment are essential to enriching and maintaining drug resistance among bacteria. At sub-inhibitory concentrations of antibiotics, bacteria do not die but their growth is slowed down. Resistance mutations caused by sub-MIC antibiotic concentrations require much less adaptation energy than mutations caused by MIC antibiotic concentrations. Therefore, mutations that incur less fitness cost could be more competitive and enriched in the microbial population [114]. There is also the minimum selective concentration (MSC) of an antibiotic, meaning the lowest antibiotic concentration that is required to select for growth of the resistant mutant [114]. In the study by Gullberg et al. [114] exploring very low concentrations of antibiotics that select resistant bacteria, the MSC value for tetracycline was 15 ng/mL and it was $1/100$ of the MIC value of the susceptible wild-type strain. For streptomycin, the MSC value ($\sim 1 \mu\text{g/mL}$) was $1/4$ of the MIC value of the susceptible strain. These results suggest that low antibiotic concentrations have a significant impact on maintaining resistance in the environment. Antibiotics at sub-inhibitory concentrations can function as signaling molecules between cells of the same or different species [115], which

has an important role in the evolution of antibiotic resistance [116]. It has been shown that antibiotics at sub-inhibitory concentrations can modulate DNA transcription [117]. The genes affected by sub-inhibitory antibiotics include genes that confer antibiotic resistance, stimulate bacterial adhesion, increase biofilm formation, and regulate mutation frequency [117]. The low doses of antibiotics may favor and sustain antibiotic resistance genes in the environment [118]. Sub-inhibitory concentrations of antibiotics affect bacterial physiology, causing mutagenesis, virulence, biofilm formation, and horizontal gene transfer (HGT) recombination. These doses can cause the occurrence of de novo resistant bacteria or enrichment of preexisting antibiotic resistant bacteria [116]. Different classes of antibiotics at sub-inhibitory concentrations affect bacterial species by inducing biofilm formation, which is a serious global health concern because it increases their ability to tolerate antibiotics, thereby enabling their survival and development [119]. Sub-inhibitory levels of imipenem, a β -lactam antibiotic, cause biofilm formation in *Pseudomonas aeruginosa* species, one of the major opportunistic pathogens [120]. A similar effect is caused by tobramycin, an aminoglycoside antibiotic [121], and norfloxacin, a fluoroquinolone antibiotic [122], which both induce biofilm formation in *P. aeruginosa* at sub-inhibitory concentrations. β -lactam antibiotics below MIC concentrations induce biofilm formation in *Escherichia coli* by inducing colanic acid synthesis, which is involved in adhesion to surfaces in this species [123]. Bacterial biofilm formation triggered by low doses of antibiotics in water favors the colonization of surfaces such as bottom sediments and solid particles found in rivers or soil. The function of antibiotics as signaling molecules also has the effect of promoting horizontal gene exchange in microbial ecosystems [115]. Antibiotics induce a bacterial SOS response to DNA damage, which regulates the horizontal transfer of integrative and conjugative elements encoding bacterial virulence, antibiotic resistance, and variety of other properties of bacterial metabolism [115]. The SOS system is a set of co-regulated genes that is extensive in bacteria and promotes cell survival by repairing damaged genomes [116]. The SOS response can increase the rate of mutation occurrence in genes conferring antibiotic resistance and increase the acquisition of antibiotic resistance [124]. SOS-inducing antibiotics include fluoroquinolones, the mode of action of which involves interaction with two target enzymes: DNA gyrase and topoisomerase IV. Resistance to fluoroquinolone antibiotics is mainly caused by point mutations in the quinolone resistance-determining region of gyrase and topoisomerase genes [125]. DNA destructive agents such as fluoroquinolones could increase HGT more than 300-fold [126]. HGT plays a crucial role in environmental dissemination of ARGs and is common in aquatic environments. This mechanism allows pathogenic bacteria to acquire antimicrobial resistance genes from the environmental gene pool (i.e., the environmental resistome) [127] by conjugation, transformation, or transduction [128]. The phenomenon of mixing drug-resistant bacteria of anthropogenic origin with environmental strains occurring in the aquatic environment increases the risk of the emergence of new antibiotic-resistant strains through HGT [129]. A second particularly essential ARG transfer element is an integron—a genetic assembly platform that can encode ARGs. The crucial integron gene encoding integrase (Int1) is induced by the SOS response. Importantly, antibiotics such as quinolone, β -lactams, and trimethoprim can induce the SOS response in bacterial cells [128]. SOS-dependent mutagenesis and horizontal gene transfer are essential factors that determine environmental antibiotic resistance and enhance the environment resistome. Antibiotics at low concentrations also act as signaling molecules that can regulate the homeostasis of microbial communities in the environment.

The presence of sub-inhibitory concentrations of antimicrobial compounds in waters causes great concern about their harmful effects on microbial community composition [130]. Direct effects of antibiotics on microbial populations might affect their abundance and species richness [131]. Antibiotics can negatively impact microbial populations involved in key ecosystem functions. Thus, they reduce biodiversity, which is crucial for maintaining the correctness of biological processes in ecosystems [130]. Importantly, sub-inhibitory levels of antibiotics can reduce bacterial community diversity by increasing the variance in fitness among taxa [132]. In a study by Cairns et al. [132] on the effect of low concentrations

of antibiotics on an experimental microbial community of 62 strains, sub-inhibitory concentrations of antibiotics were found to reduce bacterial community diversity. In addition, the diversity-reducing effect of antibiotics was lost in the presence of spatial structures (biofilms) that protected bacterial cells from the effects of pharmaceuticals. The authors suggested that determining the appropriate ecological factors (biotic and abiotic) has a significant impact on understanding the effect of sub-inhibitory concentrations of antibiotics on bacterial community composition. Zhao et al. [133] showed that levofloxacin (LEV) and oxytetracycline (OTC) at 5 µg/L sharply changed the freshwater microbial community structure at the genus level in the microcosm system without affecting the alpha diversity of the bacterial community. After seven days of LEV and OTC exposure, the relative abundance of Proteobacteria significantly increased, while that of Bacteroidetes significantly decreased at the phylum level in both treated groups. At the genus level, the abundance of Flavobacteria and Emticicia decreased while that of Pseudomonas significantly increased in the two treated groups. After 14 days of exposure to LEV and OTC, the microbial composition significantly changed at the genus level compared to the control. Changes at the genus level differed between the LEV-treated and OTC-treated groups. Flavobacteria (significantly lower after 7 days in both groups) and Niveispirilla (dominant in OTC-group after 14 days) were significantly affected by exposure to LEV and OTC antibiotics. The varying impact of the tested antibiotics on the bacterial community suggested that different bacteria in the community are sensitive to different pollutants. It should be emphasized that antibiotic residues in aquatic environments prevail as a mixture of all types of antibiotics, not as a single drug. Therefore, it can be assumed that their effects on bacterial populations will be much greater. Another example of antibiotic impact on the bacterial community composition in water is the research by Waiser et al. [134], in which the specific effects of erythromycin, trimethoprim, and clindamycin on the aquatic bacterial community composition and function of biofilm growth were reported. Erythromycin used at a concentration of 4 mg/L resulted in a bacterial community diversity in cultured biofilms that was always different from the control. Biofilm thickness and bacterial biomass were decreased after erythromycin treatment of the bacterial population. The negative impact of the antibiotics on carbon utilization was also detected. Microbial communities are potentially excellent indicators of changes in ecosystem balance, which can be disturbed by antibiotic contamination. Microorganisms play a crucial role in organic matter biodegradation and biogeochemical nutrient cycling [134]. This is particularly applicable to mountain river ecosystems, which are characterized as having much higher quality than rivers running in urbanized areas with strongly developed industry. Many rivers take their sources in the mountains and these rivers provide drinking water resources. Therefore, single changes such as antibiotic contamination can significantly disturb the stability of these bacterial communities. On the one hand, mountain areas can be characterized by significant biodiversity, while on the other hand, the compositions of bacterial populations can be unique and very sensitive to natural and anthropogenic changes [20].

5. Antibiotic Removal Processes from Water and Wastewater

Removal of antibiotics contained in wastewater (of human and animal origin) is a key aspect that could reduce the contamination of the aquatic environment (surface water and groundwater) with antibiotics. Antibiotic contamination of water creates direct and indirect routes through which antibiotics then enter human organisms. The direct route includes drinking contaminated water, while indirect routes include using contaminated water to irrigate crops that humans and livestock animals then eat or to water livestock animals that are then eaten by humans [52]. From an environmental point of view, antibiotic residues can influence microbial populations by affecting their physiological functions or can lead to the disappearance of key environmental groups of microorganisms. The problem is that conventional WWTPs are not properly prepared to remove pharmaceuticals from raw wastewater using primary treatment methods [135]. Additionally, undegraded antibiotics can adsorb onto sewage sludge in biological treatment plants. Arun et al. (2020) reported

64 ng/g of roxithromycin in the concentrated sludge of a WWTP in China [136]. This is why it is so important to reduce the amounts of antibiotics released into the environment by developing advanced wastewater treatment techniques.

Conventional WWTPs generally use primary (mechanical treatment: filtration and sedimentation) and secondary treatment processes (biological processes to remove organic matter using aerobic or anaerobic systems). The most commonly used biological method is conventional activated sludge. Membrane bioreactors (MBR) are less common [137], probably because of their high operational costs related to maintaining sustainable filtration conditions and high energy consumption [138]. The MBR process comprises aerobic and anaerobic methods, combining modern membrane filtration technology and biological degradation by active sludge. The main advantage of MBR is the high quality of the treated water suitable for its reuse [139]. Membrane bioreactors contain micro- or ultra-filtration membranes ranging from 0.04 to 0.4 μm [140], resulting in significant improvements in the microbial quality of the produced effluent by removal of a wide range of microorganisms by size exclusion [138]. Research on a pilot-scale MBR [141] showed the following percentages of antibiotic elimination from the influent from a Swiss hospital: 51% for ciprofloxacin, 47% for norfloxacin, <60% for erythromycin, 7% for sulfamethoxazole, and 96% for trimethoprim. Xiao et al. [142] showed the reduction of sulfamethoxazole and trimethoprim from wastewater using an anaerobic membrane bioreactor (AnMBR) at levels of $67.8 \pm 13.9\%$ and $94.2 \pm 5.5\%$, respectively. High removal of ampicillin (94.4%) was also achieved in MBR treatment [143]. On the other hand, the activated sludge process removed 82% of ampicillin and the disinfection process eliminated 91% of ampicillin [144] in a municipal wastewater treatment plant. MBR sewage treatment is distinguished by the increased quality of the effluent compared to conventional activated sludge systems. MBR is a compact process and its advantages are exploited in ski resorts, hotels, and trailer parks [138]. MBR effluent could be reused for technical applications, such as irrigation, snow production on ski slopes, or other non-potable water industrial applications, and not only directly discharged into the environment. Reusing water is a huge advantage in the midst of the current worldwide problem of water scarcity and water management regulations. This enables reducing the consumption of water resources and eliminating or decreasing concentrations of emerging pollutants, such as antibiotics, introduced into the environment. More developed techniques combine MBR with advanced water treatment, such as activated carbon, UV-irradiation, post-ozonation, or reverse osmosis [138,142,145]. Xiao et al. [142] achieved the elimination of sulfamethoxazole from $67.8 \pm 13.9\%$ to $95.5 \pm 4.6\%$ after adding 1 g/L of powdered activated carbon to the MBR bioreactor. Alacabey [146] achieved over 99% success in the removal of ciprofloxacin from aqueous systems with activated carbon obtained from pumpkin seed shells. Van der Waals interactions are the dominant mechanisms of organic compound removal (including antibiotics) in the activated carbon adsorption system. Carbon-based materials (activated carbon, carbon nanotubes, and graphene) are highly effective adsorbents for water-polluting antibiotics due to their large specific surface area, high porosity, and high reaction activity [147]. The efficiency of removing various compounds depends on the properties of the adsorbent (e.g., surface polarity and porosity) and the characteristics of the compound itself (e.g., size, charge, and hydrophobicity) [137]. Liu et al. [145] tested a combination of membrane bioreactor with ultraviolet/chlorine (MBR-UV/Cl₂) to treat surface water polluted with pharmaceutical personal care products and antibiotics. The average removal efficiencies of selected antibiotics from surface water in the MBR and MBR-UV/Cl₂ processes are presented in Figure 3, after supplying 200 ng/L antibiotic standards (based on [145]).

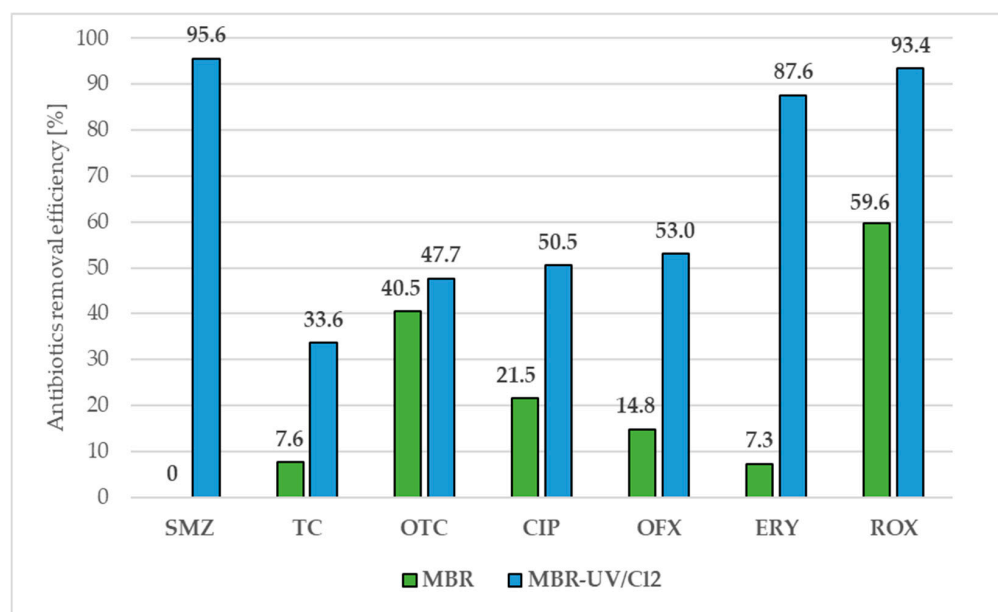


Figure 3. Antibiotic removal efficiency by MBR and MBR-UV/Cl₂ processes [143]. SMZ—sulfamethoxazole, TC—tetracycline, OTC—oxytetracycline, CIP—ciprofloxacin, OFX—ofloxacin, ERY—erythromycin, ROX—roxithromycin.

In the presented research [145], the sole MBR system was clearly ineffective in the removal of sulfamethoxazole. However, the MBR permeate subjected to additional treatment by UV irradiation and chlorination (chlorine concentration at 3 mg/L) effectively reduced 95.6% of the sulfamethoxazole contained in the polluted surface water. The UV/Cl₂ process evidently increased the antibiotic removal efficiency. Similar results of sulfamethoxazole removal (100 µg/L) were obtained by Tambosi et al. [148]. The study concerned the treatment of wastewater from a municipal treatment plant in Germany. In the MBR process, the permeate was subjected to treatment with advanced techniques such as H₂O₂/UV, H₂O₂/Fe²⁺ (Fenton), H₂O₂/Fe²⁺/UV (photo-Fenton), UV radiation, and ozone (O₃). The results showed that the elimination efficiency of sulfamethoxazole in the MBR process after 30 minutes of treatment was 64%. However, the advanced oxidation processes (H₂O₂/UV, H₂O₂/Fe²⁺/UV), UV radiation, and ozonation removed 100% of the sulfamethoxazole contained in the MBR permeates. Sulfamethoxazole was very sensitive to all of the applied UV treatment steps. On the other hand, the Fenton process was completely ineffective in the elimination of this compound (elimination rate: 0%). In the same research, the trimethoprim elimination rate by the MBR process was 94% after 30 minutes of treatment. The steps including UV treatment were less effective in trimethoprim elimination: H₂O₂/UV (7%), H₂O₂/Fe²⁺/UV (20%), UV radiation (5%), and Fenton process (10%). Ozone treatment was the most effective process that led to an elimination of 100% sulfamethoxazole and trimethoprim in the MBR permeate. High removal efficiency was also achieved for tetracycline antibiotics in anoxic/aerobic-MBR treatment for artificial wastewater containing antibiotics. The removal efficiencies after 60 days of solid retention times for tetracycline, chlortetracycline, and oxytetracycline were 93.6%, 82.9%, and 88.6%, respectively [143]. Tetracycline is one of the most frequently detected antibiotics in wastewater [149]. Dolar et al. [150] achieved excellent results in an integrated pilot scale membrane bioreactor coupled with reverse osmosis (MBR-RO) for municipal wastewater treatment. In the case of macrolide antibiotics, the MBR treatment showed removal rates of 75% for azithromycin and 87% for erythromycin. Sulfamethoxazole was partially removed (69%) with MBR treatment, whereas the removal of ofloxacin with the MBR system was ineffective in this research. Overall antibiotic removal rates with the RO membrane (pore size range < 0.001 µm) [137] were greater than 99% for each compound tested. Experimental results by Dolar et al. [150] showed that the removal performance of antibiotics was significantly higher

in MBR combined with the RO system, which effectively removed low-molecular-weight pharmaceutical compounds.

Recently, metal-organic frameworks (MOFs), a multi-dimensional material held together by bonding between metal atoms and organic ligands, have been shown to be effective in treating wastewater with antibiotic residues [151,152]. MOFs exhibit desirable characteristics, such as large surface area and pore volume, hierarchical structures, biocompatibility, non-toxicity, regeneration capabilities, and tunable pore size and functional groups, that are suitable for wastewater treatment processes [152–154]. MOFs can maintain their structures in water conditions [155]. Applying MOFs in WWTPs can significantly improve treatment efficiency. MOFs can be applied in wastewater treatment by conducting adsorption, filtration, and degradation [156], including catalytic degradation of antibiotics by immobilized enzymes [153]. Zhou et al. [157] investigated the detection and removal of tetracycline solution (0.1 mM) in water with a luminescent MOF, resulting in 56% of this antibiotic being removed after 30 minutes. Dong et al. [151] showed photocatalytic decomposition of oxytetracycline with a stable 8-connected Cd(II) MOF as a photocatalyst. Metal-organic frameworks are considered as relevant materials for the adsorption and removal of emerging pollutants, such as antibiotics, in wastewater.

Zeolites can also be used as useful materials in the treatment of antibiotics in sewage. Zeolites are sorption materials which—if appropriately developed and selectively functionalized—can retain antibiotic residues in wastewater treatment systems [158]. The hydrophobicity of zeolites is a beneficial property that facilitates the adsorption of antibiotics in water solutions. High silica-zeolites almost completely (>90%) removed sulfonamide antibiotics from water [159,160]. Natural and modified minerals are also employed in the processes of antibiotic elimination from water. They have unique properties, including high specific surface area, low cost, availability, and good removal efficiency [161]. Natural colemanite mineral (mesoporous material) was used as an adsorbent material for the removal of four common fluoroquinolones from surface water and wastewater samples. Batch adsorption experiments resulted in the following antibiotic elimination: 81.9%, 78.4%, 80.3%, and 79.7% of the initial amounts for ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin, respectively [162].

A variety of research has reported that MBR systems applying ultrafiltration are partially successful in the removal of antimicrobial agents from wastewater [142,145,148,150]. Advanced techniques combined with MBR treatment, in particular UV radiation, activated carbon, advanced oxidation processes (AOPs), and adsorption methods (MOFs, zeolites, natural materials) (Figure 4), have the potential to be developed as effective technologies in treating wastewater and surface water polluted with antibiotics. The MBR process yields treated effluent of a quality that allows it to be discharged into sensitive water bodies (such as mountain rivers) or reclaimed in a variety of ways (including the production of artificial snow). Due to its compact size, it can be used for municipal sewage treatment and can be successfully applied in places where saving space is an important trait (such as mountain hotels, shelters, etc.). It overcomes the drawbacks of conventional activated sludge processes, including large space requirements for secondary clarifiers and production of excess sludge [138,163]. The application of advanced wastewater treatment methods offers great prospects for the economical reuse of wastewater free of micropollutants such as antibiotics. Reducing the content of antibiotics in wastewater will decrease the supply of these compounds to the environment after the discharge of treated wastewater. This will reduce the risk of developing drug resistance among microorganisms in the environment and other adverse environmental effects.

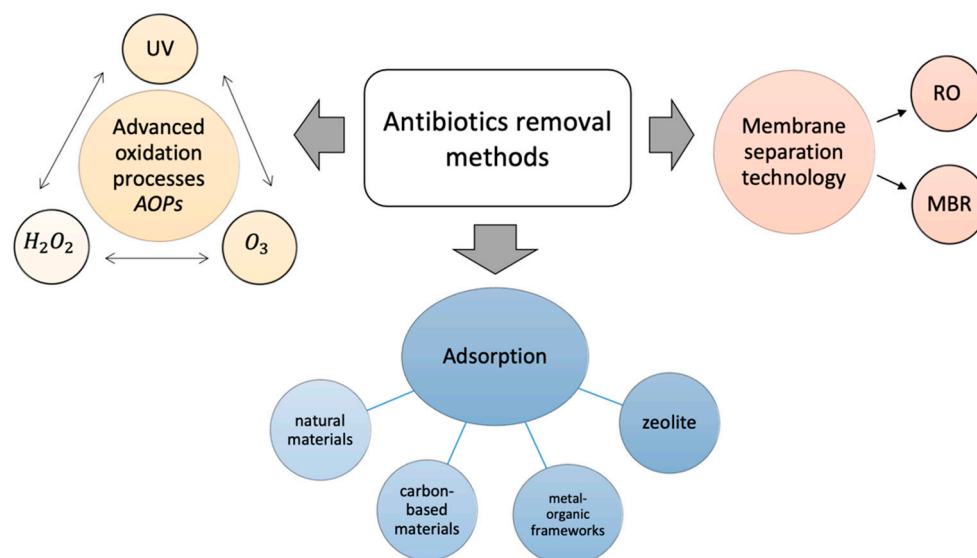


Figure 4. Scheme of advanced antibiotic removal techniques from wastewater.

6. Conclusions

Antibiotics polluting the environment are recognized as emerging micropollutants affecting microbial populations. Water is the main dissemination pathway of antibiotics and drug resistance determinants between various environmental compartments. The rate of antibiotics entering the aquatic environment is higher than their rate of elimination. Long-term exposure to sub-inhibitory concentrations of antibiotics (ng/L–μg/L) in waters is the main driver of changes in the genomes of microorganisms, thus resulting in the emergence of drug resistance and exchange of drug resistance genes by HGT. Antibiotics (acting as signaling molecules) are the ecological factor driving the evolution of bacteria by interfering with their ecological functions and compositions of bacterial communities. This causes the reduction of bacterial biodiversity responsible for the proper occurrence of biological processes in ecosystems. Antibiotics at low concentrations and bioavailability are capable of modifying bacterial communities and affect transcriptional regulation, thereby causing drug resistant mutations. Microorganisms evolve in response to emerging factors, such as antibiotics, in their environment. This is particularly evident in sensitive environments such as pristine mountain ecosystems where rivers can be exposed to strong anthropogenic factors closely related to tourism, agriculture, and animal husbandry.

The research results published so far mainly concern urban environments affected by antibiotic contamination and changes resulting thereof. However, few studies show the impact of antibiotic pollution in pristine mountain environments that are particularly sensitive to changes and under strong anthropogenic pressure resulting, among other reasons, from expansive mountain tourism. Contamination of the mountain environment with antibiotics results from intensive tourist traffic, which causes overloading of wastewater treatment plants with sewage containing micropollutants. Only partial metabolism of antibiotics in human and animal bodies after their administration is responsible for their excretion in unchanged form at levels of 5–85% and their supply in parent form to wastewater treatment plants. Conventional wastewater treatment plants using primary and secondary wastewater treatment processes are not adapted to effectively remove these micropollutants. Therefore, antibiotics end up in surface waters along with treated sewage. In mountainous areas, surface water is used to produce snow on ski slopes and irrigate crops and green areas, as well as for recreational purposes by tourists. Additional wastewater treatment with more advanced methods (MBR, UV radiation, activated carbon, membrane techniques, oxidation processes, MOFs) gives the possibility of significant or complete removal of antimicrobial agents from wastewater. The prospect of using advanced wastewater treatment techniques gives the possibility of safely reusing wastewater for technical

purposes, reducing the amount of antibiotics released into the environment, and providing an economical solution for the use of water resources.

Contamination of mountain waters with antibiotics is already present in the upper river courses of high-mountain national parks under protection. Mountain shelters, which are not equipped with sewage systems, are also sources of antibiotic contamination. There is a conflict between maintaining the pristine mountain environment and the continuous development of mountain tourism. The main threat to public health is the development of drug resistance and possible transfer of ARGs from environmental strains to clinical strains. With the continuous supply of sub-inhibitory concentrations of antibiotics in the environment affecting changes in the genomes of microorganisms, there may be a risk of a link between environmental and clinical drug resistance. On the other hand, changes in the biodiversity and composition of microbial populations that are responsible for important ecological functions in the ecosystem pose a threat to the environment. For this reason, monitoring the contamination of surface waters with antimicrobial agents is an important aspect. Contamination of surface waters with antibiotics is particularly harmful in the mountain environment. This is due to the fact that mountain water supplies are a valuable natural resource found mostly in pristine and protected areas where they give rise to rivers and constitute a reservoir of drinking water in every country. Protected environments, which are a valuable source of biodiversity, should be taken care of. Antibiotics are a type of micropollutant that is not routinely tested. Therefore, monitoring concentrations of antibiotics in waters is crucial for maintaining the quality of water resources for human use and the microbiological biodiversity within water ecosystems.

An urgent knowledge gap is the limited understanding of where and at what stage critical changes occur that affect the emergence of antibiotic resistance in bacteria and changes in bacterial population composition. Moreover, these gaps in knowledge are particularly relevant to the pristine mountain environment, where drinking water resources take their origin. Understanding the role of antibiotic contamination in the environment is important in terms of environmental changes and public health implications.

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