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Journal of Hazardous Materials Advances

journal homepage: www.elsevier.com/locate/hazadv

Microplastics in fresh- and wastewater are potential contributors to antibiotic resistance - A minireview



HAZARDOUS

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ARTICLE INFO

Keywords: Plastisphere Antimicrobial resistance Biofilm Aquatic ecosystem

ABSTRACT

Increasing antibiotic resistance and microplastic (MP) pollution are among the global environmental challenges of our time. This study reviews relevant studies on MP pollution and the abundance of antibiotic resistant elements in freshwater resources. The objective was to evaluate the potential antibiotic resistance spread via MPs in the freshwater aquatic environment. Studies have indicated that MPs interact with microbial communities differently than natural particles, harboring a unique bacterial community than the surrounding water. The composition of MP biofilm community revealed an abundance of bacterial cells, pathogens, antibiotic resistant genes (ARGs) and mobile genetic elements (MGEs). The closely packed bacterial cells in MP biofilm in a water matrix might offer a favorable environment for horizontal genes transfer (HGT). Furthermore, antibiotics in trace concentrations in freshwater resources could be adsorbed onto MP surfaces and impact the microbial composition by selective enrichment of antibiotic resistant bacteria (ARB). The biofilm matrix provide protection in MP biofilms that could impact the disinfection efficiency of conventional treatment techniques. The impacts of MPs, antibiotic resistant elements and their interaction on human health and aquatic biota remain relatively unknown to date. Future studies on MPs and antibiotic resistant elements need to consider the collective role, challenges, and consequences of the interaction between MPs and antibiotic resistant elements in freshwater ecosystem.

1. Introduction

Contaminants of emerging concern (CECs) in the aquatic environment pose potential public health challenges globally, and there is a call for research-based policies and control strategies to tackle the issue. Research studies have been conducted on identification and distribution of various CECs, including microplastics (MPs), antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in the natural environment. There is a growing number of research studies in the past few years on understanding the level of MP pollution and antibiotic resistant elements in freshwater ecosystems.

Aquatic bacterial ecology is composed of diverse bacterial species. Human activities are among major contributors of biological pollutants, including pathogen and multidrug-resistant bacteria in freshwater resources and wastewater. Armstrong et al. (1981) suggested that the multiple-ARB in drinking water distribution systems enter the wastewater stream via the sewage. The wastewater treatment plants (WWTP) effluent often enters a river, perhaps the original drinking water source, hence re-entering in the water distribution systems. Therefore, antibiotic resistance elements escaping treatment could persist and be transferred to new natural environments.

WWTPs are reported to remove significant quantities of MPs and antibiotic resistant elements. While ARGs in the WWTP effluent receiving water bodies remain on an increase, therefore WWTPs have been called hotspots for the spread of antibiotic resistance (Amos et al., 2018; Cacace et al., 2019). Similarly, although higher percentage of MPs are retained in WWTPs, the continuous effluent discharge containing escaping MPs contribute greatly to the total MP mass in the receiving water bodies. Conventional WWTPs are designed to achieve water standards based on selected physicochemical and biological parameters. The CECs removal are not regularly monitored and researches are in progress to establish the baseline and impacts associated with these pollutants.

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https://doi.org/10.1016/j.hazadv.2022.100071

Received 21 February 2022; Received in revised form 25 March 2022; Accepted 6 April 2022

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Abbreviations: ARB, Antibiotic resistant bacteria; ARGs, Antibiotic resistant genes; BPA, Bisphenol A; CECs, Contaminants of emerging concern; CFU/mL, Colony forming units per milliliter; DNA, Deoxyribonucleic acid; EPDM, Ethylene propylene diene monomer; HGT, Horizontal gene transfer; *intl1*, class 1 integron-integrase gene; MGEs, Mobile genetic elements; MPs, Microplastics; NPs, Nanoplastics; PA, Polyamide; PAN, Polyacrylonitrile; PAS, Polyarylsulfone; PE, Polyethylene; PET, Polyethylene terephthalate; PP, Polypropylene; PS, Polystyrene; PU, Polyurethane; PVC, Polyvinyl chloride; RA, Rayon; RCPE, Reinforced chlorinated polyethylene; WWTPs, Wastewater treatment plants.

Therefore, WWTPs might require improvement in designs to consider the removal of MPs, ARB, ARGs and MGEs. Additional treatment and disinfection steps might be required to acquire complete removal of all pollutants.

MPs in water bodies are considered a novel microbial niche with the potential to act as a vector for ARGs and pathogens to new environments, spreading the associated health risk across environmental compartments and the aquatic food chain (Laganà et al., 2019). Wu et al. (2019) studied the bacterial pathogens in MP biofilm and found that the MP biofilm had a distinctive resistome compared to the rock and leaf particles in the same water matrix. Furthermore, ARGs in three pathogens were found in MP biofilm only, indicating ARG enrichment by MP biofilm compared to natural particles.

In similar studies, an increased plasmid transfer had been observed among bacterial species in MP biofilm compared to those in natural aggregates. The MP biofilm indicated the potential to spread antibiotic resistance, alter aquatic bacterial communities and pose health hazards (de J.A. Andrés, 2018). Therefore, there is a possibility of MP bound pathogens acquiring ARGs from the surrounding environment and their transfer to remote environmental compartments. Previous studies have also identified opportunistic pathogens in MP biofilm (Foulon et al., 2016).

Furthermore, a potential connection is anticipated between WWTP derived bacteria and the class 1 integron-integrase gene (*intl1*) abundance in the plastisphere. It could be due to the ability of small amounts of sewage effluent to enhance the *intl1* prevalence in freshwater biofilm without alteration of the ambient water microbial community (Lehmann et al., 2016). The *intl1* gene appear in abundance in freshwater environments and wastewater. It has indicated a linkage to ARGs containing gene cassettes. Therefore, *intl1* is used as an indicator for anthropogenic pollution (Cacace et al., 2019; Power et al., 2013; Gatica et al., 2016) Therefore, mobile genetic elements (MGEs) carrying antibiotic-resistant elements in MP biofilm need attention. It is important to determine the ARG carriers in WWTP effluent and freshwater resources to determine the potential threat to human health caused by antibiotic resistance.

The present review summarizes the relevant research findings on the level and composition of MPs, antibiotic resistant elements, and their interactions in freshwater resources. Previous reviews have addressed MPs and antibiotic resistant elements in various environmental compartments, with major focus on marine environment. Two major focus of majority reviews were the analytical methods and potential health impacts. These two aspects are excluded from the present review. Furthermore, a review on the potential of MPs to lower the treatment efficacy of WWTPs, particularly the disinfection steps has not been conducted in previous review studies.

Therefore, this study attempted to answer three questions; firstly, what are the reported values of MPs and antibiotic resistant elements in freshwater resources, secondly, which are the dominant characteristics of these elements and lastly, what are the potential contributing roles of MPs in antibiotic resistance? This review contributes to the potential understanding of the movements and composition of MP biofilm in freshwater, as an opportunity to design efficient collective removal methods for MPs, ARB, ARGs, and MGEs.

2. Review methodology

A literature search was conducted on the main scientific databases by using a combination of keywords enlisted in Table 1. The title and abstract of literature from peer-review journals were screened during the primary screening step. Previous review papers were excluded from the list. Experimental studies in English language without publication date restriction were included in the review process. Literature search focused mainly on the relevance of studies to MPs and antibiotic resistant elements in freshwater environment. A detailed study of the selected literature was conducted on 34 relevant papers on MPs in freshwater

Table 1

Literature web	search	keywords.
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	Study area	Keywords
1	MPs in freshwater	(Microplastics) AND (freshwater OR wastewater OR rivers OR ponds OR glaciers OR groundwater OR lakes OR streams OR aquifers OR wetlands OR snow)
2 3	ARB/ARGs in freshwater MP biofilm containing ARB/ARGs in freshwater	Antibiotic resistance AND water (Microplastics OR plastisphere OR biofilm) AND (Antibiotic resistance bacteria OR antibiotic resistance genes OR antimicrobial resistance OR intl1) AND (water)

resources, out of it 25 papers were summarized in Table 2. Literature on antibiotic resistant elements acquired were 50, out of which 29 were summarized in Table 3 . On MP biofilm in freshwater resources, 17 papers were selected and summarized in Table 4. Characteristics of the literature consulted for the present review are available in supplementary materials (Figs. S1-S3).

3. Characteristics and occurrence of MPs in freshwater sources

Primarily originating on land, plastics have become ubiquitous in marine and freshwater sources. The degradation of plastics into microplastics and nanoplastics in marine environment have acquired much attention while in the recent few years, there has been an increasing trend in research on MPs in freshwater resources (Fig. S1). The current research trend has contributed to understanding the spatiotemporal distribution, quantities, composition, and characteristics of MP pollution in the aquatic system. Surface water exposure to human activities and atmospheric deposition have been reported as the major contributors to MP pollution in freshwater resources (Allen et al., 2019).

Li et al. (2018) and Sarijan et al. (2021) conducted a review on the occurrence of MPs in freshwater resources. Li et al. (2018) concluded that MP abundance was location dependent and the reported values were highly variable, between < 0.001 to > 1000 MPs/L. Both reviews focused on the occurrence and adopted methodologies for MP quantification. The present review addresses the MP abundance and characteristics in freshwater compartments. The summarized MP abundance provides a baseline on MP pollution. The characteristics of the MPs might aid in MP pollution source tracking and identification of MP hotspots in freshwaters.

Rivers are major freshwater sources and they may act as an intermediate plastic waste transporter between land and the oceans (Schmidt et al., 2017). Researchers have attempted to determine the role of rivers in marine plastic pollution, its potential impact on river ecosystem and on the consumers. Lebreton et al. (2017) estimated that annually between 1.15 and 2.41 million tonnes plastic waste from rivers enter the oceans. The monitoring and control of plastic waste in rivers might help mitigate marine plastic and MP pollution.

Table 2 summarizes the reported MP occurence and characteristics in various freshwater sources. The highest reported MP counted in a river system was 660 MPs/kg at a site receiving urban runoff (Horton et al., 2017). In the lake environment, the MPs in the sediments are reported to be higher than that in the water matrix (Li et al., 2019). An average quantity of MPs was > 500 MPs/kg dry sediment in Lake Ontario Canada. Furthermore, elevated number of MPs was found in lakes near urban and industrial areas (Ballent et al., 2016). Higher MP pollution in freshwater bodies located near human activities is expected, as water flow disperse pollutants while freshwater body sediments retain MPs in higher numbers.

Two most dominant MP types detected in rivers were polyethylene (PE) (Eibes and Gabel, 2021; Anon 2021; Horton et al., 2017; He et al., 2020; Kataoka et al., 2019; Lahens et al., 2018; Mintenig et al., 2020; Alam et al., 2019) and polypropylene (PP) (Anon 2021; Horton et al., 2017; He et al., 2020; Kataoka et al., 2019; Lahens et al., 2018;

Table 2

Reported abundance and characteristics of MPs in freshwater and wastewater in literature.

		Main MPs characteristics				
Water body, study location	Quantities (MPs/L)	Composition	Size (mm)	Color	Shapes	Reference
Rivers						
Minjiang River, China	6.11-44.08	***RA, PA, PP, PE	0.3	NA*	Particles, fragments, fiber	(Li et al., 2021)
River Ems, Germany	1.54×10^{-3}	PE	> 1	Blue, red	Flakes	(Eibes and Gabel, 2021)
Fenghua River, China	1.62 ± 0.88	PP	< 0.5	Transparent	Fiber	(Anon 2021)
River Thames basin, UK	660 (sediment)	PP, PE, PAS	1 - 4	NA	Fragments	(Horton et al., 2017)
Brisbane River, Australia	10 - 520	PE, PA, PP	< 3	White	Films,	(He et al., 2020)
00 Dimension	1.6	DE DD DC	. 1	274	fragments, fibers	(Veterland 1, 0010)
29 Rivers, Japan	1.6	PE, PP, PS	< 1	NA	Fragments	(Kataoka et al., 2019)
Saigon River, Vietnam	172 - 519	PE, PP, PET	0.05 - 0.25	Blue	Fibers	(Lahens et al., 2018)
Rhine River, Europe	892,777 MPs/ km ^{2**}	PS, PP, PET,	0.3 – 5	Opaque,	Fragments,	(Mani et al., 2015)
		acrylate, PCV		transparent	fibers	
2 Dutch Rivers,	0.07 – 115.3	PE, PP, EPDM	< 1	Bright,	NA	(Mintenig et al., 2020)
Netherlands				transparent		
Ciwalengke River, Indonesia	5.85 ± 3.28	PE, PA	0.05 – 0.1	NA	Fragments, forms	(Alam et al., 2019)
Lakes						
Yangtze River Basin, China	0.78 ± 0.43	РР	< 1	Blue, purple, green	Fiber	(Li et al., 2019)
Veeranam Lake, India	28 MPs/km ²	PA, PE, PVC	0.3	White, red, black	Fibers	(Bharath et al., 2021)
Lake Ontario sediment,	> 500	PE, PS, PU	< 2	Translucent,	Fibers,	(Ballent et al., 2016)
Canada				amber, black	fragments	
Dongting Lake, China	2.09×10^6 MPs/	PE, PP, PET, PS, PA,	0.33	Transparent	Fibers	(Hu et al., 2020)
Lake Guaíba Brazil	0.01 0.06		0.1 0.25	White	Fragmont	(Pertoldi et al. 2021)
Lake Guaiba, Diazii	0.01 - 0.00	rr, r£	0.1 - 0.25	transparent, red	Fragment	(Dertolul et al., 2021)
Taihu Lake, China	1.8 – 18.2	PVC, PE	< 0.1	NA	Fragment	(Zhang et al., 2021)
29 Lake tributaries, US Other freshwater resources	0.032	NA	< 5	NA	Fibers	(Baldwin et al., 2016)
Streams, Argentina	23.6×10^3	PET, PA	0.5	Blue, transparent	Fibers	(Montecinos et al., 2021)
Stormwater Denmark	0 49-22 89	PP PVC PE PET PS	> 0.5	NA	NA	(Liu et al. 2019)
Pantanal Wetlands Brazil	96+83	NA	0.05 - 0.312	Blue pink red	Fragments	(de Faria et al 2021)
Seine-centre Wastewater,	$0.26-0.32 \times 10^3$	NA	1 - 5	Transparent	Fibers	(Dris et al., 2015)
Palls	2.6	DE	01 05	214	T11	(Distance of all 0001)
wwiP influent, Italy	3.0	PE PS DE	0.1 - 0.5	NA The second se	Fibers, film	(Pittura et al., 2021)
Pyrenees mountain catchment, France	$365 \pm 69 \times 10^{\circ}$ MPs/km ²	PS, PE	≤ 0.05	Transparent, orange, blues, greens, purples, black	fibers, film	(Allen et al., 2019)
Arctic Central Basin	7×10^{-2}	PET, PA, PAN	1 - 5	Blue, transparent	Fibers, fragments	(Kanhai et al., 2018)
Aquaculture, China	0.12 - 0.53	PE, PP, PET, PS, PA, PVC	0.1 – 0.5 & > 1	NA*	Fragment, fibers	(Xiong et al., 2021)

NA* Not available, Km^{2**} Reported per surface area, ***Refer list of acronym for abbreviations.

Mintenig et al., 2020; Mani et al., 2015). The reported sizes were between 0.05 to 5 mm. Eibes and Gabel (2021) reported flakes dominating among MPs in River Ems Germany, while other reported shapes were fibers (Anon 2021; He et al., 2020; Lahens et al., 2018; Mani et al., 2015), fragments (Horton et al., 2017; He et al., 2020; Kataoka et al., 2019; Lahens et al., 2018; Mani et al., 2015) among others (Table 2). The more frequently reported MP were blue (Eibes and Gabel, 2021; Lahens et al., 2018) and transparent (Anon 2021; Mintenig et al., 2020; Mani et al., 2015). The MP pollution source determined the MP types in the river systems, for instance, rayon (RA) and polyamide (PA) with larger sizes and fiber shape dominated urban areas, while PP and PE in rivers were higher near agricultural areas (Li et al., 2021). Therefore, the characteristics of MPs in freshwater bodies might provide information on the origin of MP pollution and the parent plastic material degraded into MPs. For instance, Lusher et al. (2015) suggested that higher fibers among particles indicates their origin from the breakdown of larger items and/or input from wastewaters. Alternatives for plastic types contributing the most to MPs in freshwater bodies may help reduce MP pollution.

In lakes, PE (Ballent et al., 2016; Bharath et al., 2021; Bertoldi et al., 2021; Zhang et al., 2021), PP (Li et al., 2019; Bertoldi et al., 2021)

and polyvinyle chloride (PVC) (Bharath et al., 2021; Zhang et al., 2021) appeared frequently. Dominant MP sizes were between < 0.1 to 5 mm (Table 3). Prevailing MP shapes reported were fibers (Li et al., 2019; Ballent et al., 2016; Bharath et al., 2021; Xiong et al., 2021; Hu et al., 2020; Baldwin et al., 2016) and fragments (Ballent et al., 2016; Bertoldi et al., 2021; Zhang et al., 2021; Xiong et al., 2021). The highly reported MP color in lakes were, transparent (Bertoldi et al., 2021; Hu et al., 2020), red (Bharath et al., 2021; Bertoldi et al., 2021) and black (Ballent et al., 2016; Bharath et al., 2021). MPs in black color were attributed to black tire rubber (Lenz et al., 2015). Similarly, most of the transparent MPs were identified as polystyrene sulfonate (PSS) in another study (Ballent et al., 2016). Evangeliou et al. (Evangeliou et al., n.d.) simulated that 34% of tire wear and 30% of the brake wear particles deposit in the World Ocean through atmospheric transport. The appearance of black MPs might indicate towards exposure to road dust deposit in freshwater bodies or storm water discharge from pathways into freshwater bodies.

MPs are not only concentrated in rivers and lakes. For instance, the reported MP quantity in freshwater aquaculture was 0.5 MPs/L. The majority of MPs were in sizes between 0.1–0.5 mm and > 1 mm (Xiong et al., 2021). MPs in streams were characterized by polyethy-

Table 3

Abundance and characteristics of antibiotic resistant elements in freshwater and wastewater.

Antibiotic resistance profile					Analysis	
Water body, Location	Antibiotics (ng/L)	ARB (CFU/L)	ARGs (copies/L)	MGEs (copies/L)	techniques	Reference
Drinking water & sources Mineral water, Portuguese and French brands	NA	up to 10 ⁵ (isolates resistant to 22 microbial inhibitors)	NA	NA	Plate-screening method	(Falcone-Dias et al., 2012)
Tap water, Poland	NA	Ceftazidime resistance	blaTEM, tetA, sull, ermB, qacED1, qacH, tnpA	Intl1	Plate-screening, PCR, Denaturating gradient gel electrophoresis	(Siedlecka et al., 2020)
Tap water, London UK	NA	Vancomycin, erythromycin, amoxicillin and trimethoprim resistant ARB	sul1, tet(A), bla-TEM1, mph(A) and dfrA7	NA	Plate-screening, PCR	(Destiani and Templeton, 2019)
Reclaimed water distribution system, Saudi Arabia	NA	NA	<i>adeF</i> and <i>qacH</i> at point-of-use	NA	Metagenomics analysis	(Wang and Hong, 2020)
Chlorine disinfected drinking water, China	NA	Chloramphenicol, trimethoprim and cephalothin resistant ARB	sul1, tetA, tetG, ampC, aphA2, bla(TEM-1), ermA, ermB	Intl1	Metagenomic analysis, qPCR	(Shi et al., 2013)
Drinking water (non-disinfected), China	7.9 (13 antibiotics)	NA	3.45 × 10 ⁵ (sul 1, sul 2, qnr S, qnr D, tet C, tet G, erm A, erm B)	4.24–4.23 × 10 ² (<i>Intl1</i>)	UPLC-MS/MS, qPCR	(Gu et al., 2021)
Drinking water (non-disinfected), US	NA	Enterobacter cloacae, Klebsiella pneumoniae, Escherichia coli, Pseudomonas, Enterococcus, Staphylococcus, Bacillus spp	sul1 and tetA	NA	Kirby-Bauer Method, qPCR	(Bergeron et al., 2015)
Drinking water source rivers, China	161.25 - 472.42 (20 antibiotics)	NA	2.72×10^{6} and 1.54×10^{8} (sul1 and erm36)	2.79×10^{8} , 6.23×10^{7} , and 1.51×10^{7} (Intl1)	qPCR, LC-MS/MS	(Liu et al., 2021)
Drinking water source, China	19.68 - 497 (10 antibiotics)	NA	6.5 × 10 ⁷ - 1.6 × 10 ⁹ (18 ARGs)	Intl1 (6.5×10^7 - 1.6×10^9)	UPLC-MS/MS, qPCR	(Hu et al., 2021)
Drinking water source, China	NA	NA	10 ⁻⁴ –10 ⁻³ (ARGs/ <i>16S rRNA</i>)	8.0 × 10 ⁻² (<i>intl1/16S</i>)	qPCR	(Hu et al., 2021)
River Rhine, Germany	NA	Resistance to amoxicillin, trimethoprim, sulfamethoxazol, tetracycline	bla(TEM), bla(SHV), ampC, sul1, sul2, dfrA1, tet(A), tet(B)	Intl1, intl2	Disk-diffusion method and PCR of 100 <i>E. coli</i> isolates	(Stange et al., 2016)
Kshipra River, India	2750 (sulfamethoxazole residues)	Ampicillin, cefepime, meropenem, amikacin, gentamicin, tigecycline, multidrug resistant ARB	blaCTX-M-1	NA	HPLC- MS, PCR, Kirby-Bauer Method	(Diwan et al., 2018)
Yangtze River, China	NA	Dominant ARB phylum, <i>Proteobacteria</i> and <i>Firmicutes</i> . Resistance to ampicillin, streptomycin, rifampicin, chloramphenicol, kanamycin	NA	NA	Plate-screening, 16 s rDNA sequencing	(Bai et al., 2015)
Poyang River, China	sulfonamides	NA	sul1, sul2, sul3, tetA, tetB, tetC, tetH, tetW, tetO, tetM, qnrS, qnrB	Intl1 detected	HPLC- MS, PCR, qPCR	(Liang et al., 2020)
River systems, Germany	600 (clarithromycin), 400 (sulfamethox- azole) and 390 (trimethoprim)	multidrug-resistant bacteria, <i>E. coli</i> and <i>Enterococcus faecium</i>	bla(OXA-58)	NA	HPLC-MS, plate-screening, MALDI-TOF MS, qPCR	(Voigt et al., 2020)
03 Recreational lakes, China	NA	Resistance to penicillin-G, ampicillin, vancomycin, erythromycin, ceftriaxone, gentamycin, tetracycline, chloramphenicol.	10 ⁶ (<i>blaTEM</i> gene was the most prevalent)	NA	Plate-screening, qPCR	(Fang et al., 2018)
River & lake, Greece	NA	Abundant Streptomycin resistance. ARB susceptible to amoxycillin-clavulanate, cefuroxime, ciprofloxacin, colistin, amikacin and apramycin	NA	NA	Kirby-Bauer Method, 20 microbial tested on 79 Salmonella strains.	(Arvanitidou et al., 1997)

(continued on next page)

Table 3 (continued)

Water body, Location		Antibiotic resista	Analysis	Reference		
	Antibiotics (ng/L)	ARB (CFU/L)	ARGs (copies/L)	MGEs (copies/L)	techniques	
Qingcaosha reservoir, China	Sulfamethoxazole, sulfamonomethox- ine and penicillin G potassium salt	NA	3.08×10^{-2} - 1.12×10^{-1} (sul1/16 s) and 4.12×10^{-3} - 2.99×10^{-2} (sul2/16 s)	4.21 × 10 ⁻¹ (<i>Intl1/16 s</i> rRNA)	LCMS, qPCR	(Xu et al., 2020)
Qingcaosha Reservoir, China	NA	NA	$3.7 \times 10^6 - 1.4 \times 10^9$ (sul1, sul2 most abundant)	Intl1 ($9.5 \times 10^6 - 4.0 \times 10^9$)	qPCR	(Huang et al., 2019)
Other water sources						
Groundwater, China	NA	NA	sul1 & sul2	intl1 and tnpA-04	HT-qPCR	(Wu et al., 2020)
Aquaculture, China	98.6 (09 antibiotics)	NA	2.8×10^{-2} (ARG/ <i>16s</i> - for 15 ARGs)	NA	UP-LC MS, PCR, qPCR	(Xiong et al., 2015)
Irrigation ditches, Spain	NA	Multi-resistant E. coli	blaTEM, qnrS, tetW, sul1 and ermB	NA	Plate-screening, qPCR	(Amato et al., 2021)
Constructed wetland, China	0.15 to 59.52 (21 antibiotics)	NA	2.41×10^{-4} - 1.87 × 10 ⁻² (<i>TetG</i> , <i>tetX</i> and <i>sul2/16 s</i>)	$3.40 \times 10^{-1} - 2.77 \times 10^{-1}$ (<i>Intl1</i>)	UHPLCMS/ MS, qPCR	(Du et al., 2021)
120 Bathing areas, Greece	NA	Resistant to erythromycin, ciprofloxacin, rifampicin, kanamycin, streptomycin in enterococcal isolates.	NA	NA	Kirby-Bauer Method	(Arvanitidou et al., 2001)
Coastal water recreational, US	NA	Resistant to carbapenem, monobactam, penicillin, tetracycline, sulfonamide, cephalosporin	erm(B), Sul1, tet(A), tet(W)	NA	Kirby-Bauer Method, qPCR	(Belding and Boopathy, 2018)
WWTPs						
Hospital wastewater, Spain	13,780 (ciprofloxacin), 14,380 (Ofloxacin), metronidazole, sulfamethoxazole & trimethoprim	NA	blaTEM, qnrS, ermB, sull, tetW	NA	UPLC, qPCr	(Rodriguez-Mozaz et al., 2015)
04 WWTP effluent, China	1441.6–4917.6 (10 antibiotics)	NA	1–10 (08 ARGs). <i>sul1</i> & <i>sul2</i> , abundance.	NA	UPLC-MS-MS, qPCR	(Wang et al., 2021)
Treated wastewater, Antarctic	890 and 750 (08 antibiotics)	Trimetropim and nalidixic acid -a first generation quinolone ARB	NA	NA	LC-MS/MS, disk diffusion method	(Hernández et al., 2019)
16 WWTP effluent, EU	NA	ŇĂ	Most abundant ARG was <i>Sul1</i> . 6 out of 9 ARGs detected	Intl1	qPCR	(Cacace et al., 2019)

lene terephthalate (PET) and PA of dominant size 0.5 mm in blue and transparent colors. Fibers appeared higher than other MP shapes (Montecinos et al., 2021). Stormwater draining into freshwater systems was found as a contributor to MP pollution. In one such study, collected stormwater contained 0.5 - 22.9 MPs/L, with diverse composition of PP, PVC, PE, PET, polystyrene (PS) in sizes > 0.5 mm (Liu et al., 2019). In wetlands, a study reported MP abundance of 9.6 MPs/L, in size range of 0.2 ± 0.1 mm in blue, pink, and red color fragments (de Faria et al., 2021). These studies indicated that various MPs are scattered across freshwater ecosystems. The variety in MP color in freshwater bodies are predicted to attract smaller aquatic animals searching for food. The MP size in the range of plankton size makes them suitable for consumption by aquatic invertebrates (Browne et al., 2008). While the MPs associated ecological and health risks remains relatively unknown.

Wastewaters are expected to contain high MPs from domestic and industrial sources. WWTP influent contained 3.6 MPs/L with major characteristics as PE in fibers and film shapes, in 0.1–0.5 mm size (Pittura et al., 2021). In another study, the MPs appeared in size ranges of 1–5 mm, dominated by transparent fibers (Dris et al., 2015). MPs have also been detected in remote water sources. For example, due to rare human activities in the Arctic, MPs were anticipated to have a minimal presence, but recent studies revealed 0.007 MPs/L/day in the subsurface water of the Arctic Central Basin, constituted by PET, PA, and

polyacrylonitrile (PAN) in size range of 1- 5 mm. Blue and transparent fibers and fragments were among the MPs (Kanhai et al., 2018). MPs were also detected in mountain catchment areas. The source of MP at remote locations was attributed to atmospheric transport and deposition of MPs (Allen et al., 2019).

MPs distributed in freshwater resources are dependent on land sources, air and water transports of the MPs. The characteristics of MPs in different freshwater resources might aid in tracing the target point source for the purpose of planning MP removal. From all these data, summarized in Table 2, it can be concluded that the highest MP numbers are reported in rivers and lakes, the two major drinking water sources. MPs in freshwater appeared irrespective of the location, both developed and developing countries have reported MPs in freshwater resources. The proximity of the water body from human activities might contribute to MP abundance but MP pollution appeared as a global issue.

4. Prevalence of antibiotic resistance elements in freshwater sources

Antibiotics, ARB, and ARGs have been found in freshwater ecosystems. Major research studies conducted in the last decade have concentrated on the detection of antibiotic resistant elements in drinking water

Table 4

Studies on microplastic biofilm harboring antibiotic resistant elements in water sources.

Location	Water matrix	ARB / bacterial analysis	Dominant ARGs / mobile elements	MPs	Reference
Lab-scale	Treated wastewater	Whole community	<pre>aac(6')-Ib-cr, drfA1, ermB, ermF, mefA, sul1, sul2, tetA, tetX, intl1</pre>	PVC, PE, PET	(Zhang et al., 2021)
Lab-scale	Ultrapure water	E. coli	NA	PE, PA	(Shen et al., 2021)
Lab-scale	Wastewater & river water	NA	BacHum, sul1	PE and PS	(Parrish and Fahrenfeld, 2019)
China	Watershed	Whole community	sul1, sul2, tetA, tetB, tetM, tetW, qnrB, qnrS, ermB, ermF, intI1	PE, PP, PBD	(Hu et al., 2021)
Italy	Treated wastewater	Whole community	tetA, sul2, ermB, qnrS, blaCTXM, intI1, MRG	PE, PP, PS, PET, PAN, silicon	(Galafassi et al., 2021)
China	Urban water	Whole community	aadA7, cphA, qepA, lnu(F), qacH, sul1, intI1, IS26, ISPps1-pseud, pBS228-IncP-1, trb-C	HDPE	(Yang et al., 2020)
China	Treated wastewater	NA	intl1, tetE	PVC	(Dai et al., 2020)
China	River, estuary, and marine waters	Whole community	<pre>sul1, sulA/folP, tetA, tetC, tetW, tetX, ermE, ermF, cmlA-02, intl1</pre>	PE	(Wang et al., 2020)
China	Sewage	Legionella, Mycobacterium, Neisseria, Arcobacter	Multidrug, tet, bla	PE, PVC	(Wang et al., 2021)
China	Municipal wastewater	E. coli	tet	PE, PP, PS and RCPE	(Cheng et al., 2022)
US	3 WWTPs	Whole community	sul1, sul2, intl1	PE, PS	(Pham et al., 2021)
US	2 WWTPs	Whole community	NA	PE, PP, PS	(Kelly et al., 2021)
China	Landfill leachate	Whole community	sul1, sul2, ermB, mefA, aadA1, strB, blaOXA, blaTEM, tetQ, tetM, intl1, intl2, traA, trbC	PS	(Shi et al., 2021)
China	Estuary	Whole community	sul1, sul2, tetA, tetO, tetW, Chl, aac(6')-Ib	PP, PE, PET, PS, PVC	(pan Guo et al., 2020)
China	Mariculture pond	Whole community	<pre>floR, sul1, tetG, mfpA, bacA, intl1, groEL/intl1, intl9, intlA, orf/intl1, intl2</pre>	PET	(Lu et al., 2022)
China	Aquaculture	Whole community	tetG, qnrS, sul1, sul2, ermF	PET	(Lu et al., 2019)
China	Mariculture pond	Vibrio, Muricauda, Ruegeria	tetA, tetB, tetC, tetD, tetE, tetG, tetK, tetL, tetM, tetO, tetQ, tetS, tetT, tetW, tetX, sul1, sul2, sul3, qnrA, qnrB, qnrS, cmlA1, cmx(A), ampC, blaCTX-M, blaSHV, blaTEM, aac(6')-Ib, aacA/qphD, aacC2, aacC4, aadA1, aphA3, str, creA, creB, mphA, multiduus	PET	(Zhang et al., 2020)

sources, reservoirs, and WWTP effluents (Fig. S2). Various studies have indicated that WWTPs are unable to remove these elements completely. In a study on 57 WWTPs effluent across 22 countries, over 10^6 CFU/L cefotaxime-resistant coliform was frequently detected (Marano et al., 2020). Voigt et al. (2020), termed WWTP effluent as point sources for ARB. Moreover, Bergeron et al. (2017), found the filtration systems ineffective against the removal of bacterial DNA upon detection of 16srRNA consistently in raw, finished, and tap-water throughout the year.

WWTPs contributed to the enhancement of the ARGs and intl1 abundance, indicating potential horizontal gene transfer (HGT) in treated waters (Lupan et al., 2017). Furthermore, antibiotics have been detected in source wastewaters, drinking water sources, and at point-ofuse. The concentration of antibiotics reported in various freshwater resources are summarized in Table 3. The findings indicate the prevalence of antibiotic resistant elements across freshwater resources. Appearance of these elements in treated waters further indicate the failure of conventional WWTPs to remove all ARGs. WWTPs have been called hotspots of antibiotics and ARGs. The reported removal range is between 60- 100% antibiotics and 2 - 4.6 logs removal of ARGs in multiple studies (Rizzo et al., 2013; Michael et al., 2013; Cai et al., 2018; Le-Minh et al., 2010; Gao et al., 2012). Moreover, the prevalence of antibiotics in the aquatic environment indicate their environmental persistence and extensive use in medical treatments (Carvalho and Santos, 2016; Zhang et al., 2015; S. Li et al., 2018; Anon 2003).

Similarly, the most frequently reported ARGs in hospital wastewater were those resistant to beta-lactam (*blaTEM*), quinolones (*qnrS*), macrolide (*ermB*), sulfonamide (*sul1*) and tetracycline (*tetW*) (Rodriguez-Mozaz et al., 2015) class of antibiotics. Treated water are reported to contain various ARGs and MGEs. For instance, ARB (Hernández et al., 2019), *intl1* and *sul1* (Cacace et al., 2019; Wang et al., 2021) were detected in abundance in WWTP effluent. Multi-resistant *E. coli* and ARGs were isolated from irrigation ditches and groundwater (Amato et al., 2021; Wu et al., 2020). Antibiotic resistant elements found in coastal recreational sites and bathing areas included ARB, *ermB, sul1, tetA, tetW* (Belding and Boopathy, 2018; Arvanitidou et al., 2001). Drinking water sources, rivers, lakes and reservoirs contained abundant ARB (Voigt et al., 2020; Bai et al., 2015; Arvanitidou et al., 1997; Diwan et al., 2018) and the most prevalent ARGs were *blaTEM* (Fang et al., 2018; Stange et al., 2016), *sul1* (Stange et al., 2016; Liang et al., 2020; Xu et al., 2020; Liu et al., 2021; Huang et al., 2019), *sul2* (Stange et al., 2016; Liang et al., 2020), *tuet al.*, 2020; Huang et al., 2016; Liang et al., 2020) among others (Table 3).

Drinking water and bottled mineral water were reported to contain antibiotic resistant elements. Mineral water contained up to 10^2 CFU/mL of ARB (Falcone-Dias et al., 2012). Various other ARB, ARGs, and *intl1* were reported in chlorine disinfected water (Shi et al., 2013) and tap-water (Destiani and Templeton, 2019; Siedlecka et al., 2020). Despite the reported concentrations of antibiotic resistant elements in waters, improved removal techniques, the exposure level, and associated health risk remain understudied to date.

Diverse ARGs have been detected in water environment, indicating a potential antibiotic resistance to multiple antibiotics (Table 3). Similarly, the reported multiple antibiotic resistance mechanism has not been fully understood to date, requiring further studies (Mudryk, 2002). While, MGEs (transposons) are associated with the exchange of ARGs, among plasmids and bacterial chromosomes (Herwig et al., 1997).

5. Potential contribution of MPs to antibiotic resistance proliferation

In the last few years, there has been a growing concern over the interaction of MPs with other pollutants, including antibiotics, ARB, ARGs, and MGEs (Fig. S3). Studies have indicated that these contaminants of emerging concern (CECs) are distributed across freshwater bodies in



Fig. 1. Visual representation of MP surface (potential binding sites for antibiotic residues, ARB, ARGs, and MGEs) before and after incubation in WWTP effluent.

abundance (Table 2, Table 3). The co-occurrence of MPs and antibiotic resistant elements might pose additional challenges due to various potential roles MPs might assume, owing to its physical properties and ubiquity.

Bacterial colonization on MP surface has attracted major attention from the scientific community. Biomolecules, organic matter and microbes attach to the hydrophobic surfaces in water matrix. As high has 99% of bacterial population attach to surfaces during different bacterial growth stages (Dalton and March, 1998). The MP characteristic and environmental factors affecting MP biofilm formation in the aquatic environments have been evaluated in a review conducted by Yang et al. (2020). Elgarahy et al. (2021), reviewed the MP prevalence in aquatic environments. They concluded that research findings indicate towards potential health impacts associated with MPs. MPs interation with organic/inorganic pollutants and its accumulation in the ecosystem expose aquatic organisms and humans to the pollutants.

Kaur et al. (2021) conducted a similar review on MP associated pathogens and antimicrobial resistance in the environment. They indicated the potential role of antibiotics in water bodies to induce antimicrobial resistance in MP bound microbes. While their review summarized findings on MPs interaction with pathogens and ARGs in the natural environments and detailed the MP biofilm analysis methods, the present study reviewed specific roles of MP in antibiotic resistance proliferation in freshwater resources (Fig. 1).

5.1. MPs associated antibiotic-resistant microbiota and genes

Geyer et al. (2017) suggested that MPs could act as a reservoir of antibiotic resistant elements for long-term. Various other studies have indicated that the MP biofilm community is unique from the surrounding water (Parrish and Fahrenfeld, 2019). Research studies have compared the bacterial community and ARGs abundance in the MP biofilm with the surrounding water (Table 4). MP biofilms were studied in wastewater (Wang et al., 2021; Cheng et al., 2022), treated wastewater (Parrish and Fahrenfeld, 2019; Dai et al., 2020; Zhang et al., 2021; Galafassi et al., 2021), river water (Parrish and Fahrenfeld, 2019; Wang et al., 2020), estuary (Wang et al., 2020; pan Guo et al., 2020), watershed (Hu et al., 2021) and aquaculture (Lu et al., 2019).

MP biofilm indicated a diverse bacterial community (Wang et al., 2021; Zhang et al., 2021; Galafassi et al., 2021; Wang et al., 2020; pan Guo et al., 2020; Hu et al., 2021; Lu et al., 2019; Yang et al., 2020; Pham et al., 2021; Kelly et al., 2021), ARB (Wang et al., 2021) and *E. coli* (Cheng et al., 2022; Shen et al., 2021). MP biofilm contained ARGs resistant to antibiotic classes: aminoglycoside (Zhang et al., 2021; pan Guo et al., 2020; Yang et al., 2020), trimethoprim (Zhang et al., 2021), macrolide (Zhang et al., 2021; Galafassi et al., 2021; Wang et al., 2020; Hu et al., 2021; Galafassi et al., 2021; Wang et al., 2021; Cheng et al., 2022; Dai et al., 2020; Zhang et al., 2021; Galafassi et al., 2021; Wang et al., 2020; pan Guo et al., 2020; Hu et al., 2021; Lu et al., 2021; Wang et al., 2021; Lu et al., 2020; Hu et al., 2021; Lu et al., 2021; Wang et al., 2021; Lu et al., 2021; Wang et al., 2021; Lu et al., 2020; Hu et al., 2021; Lu et al., 2021; Wang et al., 2021; Lu et al., 2020; Hu et al., 2021; Lu et al., 2020; Hu et al., 2021; Lu et al., 2021; Wang et al., 2021; Lu et al., 2021; Wang et al., 2021; Lu et al., 2020; Hu et al., 2021; Lu et al., 2019), sulfonamide (Parrish and Fahrenfeld, 2019; Zhang et al., 2021; Lu et al., 2021; Lu et al., 2021; Zhang et al., 2021; Lu et al., 2021; Lu et al., 2021; Lu et al., 2021; Zhang et al., 2021; Lu et al., 2021; Lu et al., 2021; Zhang et al., 2021; Zhang et al., 2021; Zhang et al., 2021; Zhang et al., 2021; Lu et al., 2021; Zhang et al., 2021;

Galafassi et al., 2021; Wang et al., 2020; pan Guo et al., 2020; Hu et al., 2021; Lu et al., 2019; Yang et al., 2020; Pham et al., 2021), quinolones (Galafassi et al., 2021; Hu et al., 2021; Lu et al., 2019; Yang et al., 2020), beta-lactam (Wang et al., 2021; Galafassi et al., 2021), multidrug (Wang et al., 2021; Galafassi et al., 2021; Yang et al., 2020), metallobeta-lactamase (Yang et al., 2020), fluoroquinolone (Yang et al., 2020) and chloramphenicol (Wang et al., 2020). It is evident from the studies that MPs accumulate diverse types of ARGs in freshwater aquatic environment.

MPs contained distinct bacterial species whose abundance was comparable to wood particles but significantly higher than water and sediment. *Pseudomonas, Flavobacterium, Rhodoferax, Pseudomonas,* and *Janthinobacterium* were enriched on the MPs, indicating their potential preference for selected species. While relative abundance of total ARGs did not vary significantly across the water, sediments, wood particles, and MPs (Hu et al., 2021), only 4 ARGs were targeted, limiting generalization. Finally, MPs harbored significantly different bacterial communities at phylum and genus levels than aquaculture water (Lu et al., 2019).

In pre- and post- disinfected water, MP bacterial community was found to be different in richness, structure, and composition from the surrounding water. While in ozone disinfected water, relatively lower potential contribution of MPs to resistance spread and pathogenic species propagation was predicted, only *sul1* gene was more abundant in MP biofilm (Galafassi et al., 2021). The finding of this study might not hold true for other disinfection methods, due to the varying potential impact of disinfection techniques on the microbial community.

In a WWTP, the addition of MPs significantly increased the bacterial diversity and richness. The results indicated a positive correlation between MPs and ARGs (Dai et al., 2020). In another study, a comparison of MP biofilm in WWTPs showed a modified MP biofilm bacterial community upon treatment in the effluent (Kelly et al., 2021). Parrish and Fahrenfeld (2019) conducted batch experiments with natural river water and wastewater to evaluate the factors affecting the MP biofilm associated bacterial community. They concluded that water quality and MP type are factors affecting the bacterial community on the MP surface, while the size of MPs had non-significant effect.

5.2. MPs facilitate ARGs exchange via MGEs

Biofilms as hotspot for HGT in aquatic environment was reviewed by Abe et al. (2021). They elaborated the mechanisms involved in ARG exchange within biofilms. The HGT depends on the bacterial communities in the biofilm, surrounding environmental conditions and MGEs. Additional to the known HGT mechanisms of conjugation, transduction and transformation, they indicated that a new interspecies HGT mechanism called membrane vesicles might play role in ARG exchange. The review did not take into consideration the MP biofilm. The DNA exchange through membrane vesicles in MP biofilm remained to be studied.

MP biofilm was found to contain an abundance of MGEs in freshwater sources. The presence of MGEs and ARGs among the bacterial community provides a viable environment for exchanging genetic elements in MP biofilm. Among MGEs, the most frequently studied and encountered gene was *intl1*, which appeared in the MP biofilm in WWTPs (Pham et al., 2021), treated wastewater (Dai et al., 2020; Zhang et al., 2021; Galafassi et al., 2021), watershed (Hu et al., 2021), rivers, and estuaries (Wang et al., 2020). MP biofilm in urban waters studied for MGEs indicated the presence of *intl1*, *IS26*, *ISPps1-pseud*, *pBS228-IncP-*1, and *trb-C* (Yang et al., 2020).

Furthermore, Zhang et al. (2021) reported that MPs amplified the *intl1* gene in a sludge digestion system. MPs also selectively enriched bacterial hosts, indicating potential for HGT. Wang et al. (2021) reported enrichment of MGEs in MP biofilm, that persisted in the presence of supplemented antibiotics in the sewage. In municipal wastewater, 4 types of MPs (PE, PP, PS and RCPE) indicated accumulation of extracellular ARGs significantly higher than the intracellular ARGs. The obser-

vation potentially indicates towards improved HGT by MPs, attributed to a better contact environment between donor and recipient bacterial cells. On MP surface, an improved permeability and ARGs abundance was observed in ARGs receiving bacteria (recipient cells) (Cheng et al., 2022).

Arias-Andres et al. (2018), also demonstrated an amplified antibiotic resistant gene carrier plasmid transfer within the MP biofilm than that of the surrounding water and natural aggregate associated microbial community. Furthermore, the study indicated the occurrence of HGT in broader and diverse bacterial phylogeny.

5.3. MPs are selective enrichment hubs for antibiotic resistant elements

In a review conducted by Liu et al. (2021), studies supported the selective enrichment of antibiotic resistant elements on MP surface in various environmental compartments (aquatic, terrestrial, air, WWTPs and landfill leachate). They indicated that the distinct ARG composition of MPs than the surrounding environment is due to selective enrichment of ARGs and ARB. In another review, Syranidou and Kalogerakis (2022) evaluated findings on the interations among MPs, antibiotics and ARGs in WWTPs. They concluded that MPs interact with antibiotics by hydrophobic, electrostatic and intermolecular interactions. Antibiotic pressure is a potential contributor to enhanced ARG expression and selective ARB survival among bacterial community.

An increased abundance of potential bacterial hosts and ARGs were observed by Zhang et al. (2021), with the addition of MPs in an aerobic sludge digestion system. They attributed it to selective enrichment of ARGs and bacterial hosts by MPs in WWTPs. Furthermore, in a similar study, Dai et al. (2020) reported a positive correlation between MPs and ARGs. Batch experiments conducted with real samples of marine water, rivers, and estuaries showed selective enrichment of microbe, ARGs, and antibiotics on PE MPs in the studied water matrix. Moreover, microbial diversity and ARGs on MP surfaces showed elevated values in the river water.

It was observed that under antibiotic pressure, new ARGs were expressed in the MP biofilm (Wang et al., 2020). Similar observations were made by Wang et al. (2021), where ARGs and pathogenic bacteria appeared in more abundance on MPs surface than in the surrounding sewage itself, making MP biofilms repositories of ARGs. The study also reported that the antibiotic pressure induced amplification of ARGs in MP biofilm in sewage.

These results are supported by batch experiments with seeding activated sludge from three WWTPs. In the study, Pham et al. (2021) demonstrated the potential of MPs (PE and PS) to harbor and promote ARB (*Raoultella* and *Stenotrophomonas* sp.), ARGs (*sul1* and *sul2*), and MGEs (*intl1*) selectively. The selective enrichment was not observed in the control reactor with fine sand without MPs. The addition of antibiotics (sulfonamide) indicated a 1.2- to 4.5-fold increment in the absolute number of ARGs in the MP biofilm.

5.4. MPs act as a vector for antibiotic resistant elements

MP biofilm selectively enrich the ARGs. MPs that discharge in the effluent transport the ARGs from WWTPs to the receiving freshwater bodies. Sathicq et al. (2021) reviewed the literature to evaluate if MPs act as a hotspot for the spread of antibiotic resistance in aquatic systems. They indicated that both MPs and ARB originates from human sources, sharing pollution sources. They concluded that further studies are needed to fully evaluate health impacts of MPs role as a carrier for ARB and ARGs.

MP surfaces could adsorb antibiotics and transport them to other environmental compartments. Atugoda et al. (2020) showed that PE MPs act as a potential vector for ciprofloxacin in aquatic environments, influenced by MP properties, pH, ionic strength, and dissolved organic matters in the water. MP size had appeared to play a minimal role in determining the MP biofilm microbial community (Parrish and Fahrenfeld, 2019). Macro-plastic recovered from the Antarctica indicated the presence of biofilm forming bacterial species and multidrug resistant strains against cephalosporins, quinolones, and beta-lactams (Laganà et al., 2019). The coexistance of antibiotic resistant elements and MPs in remote regions indicate towards the transport of these elements on MP surface. Atmospheric deposition of MPs in remote regions might contribute to the spread of antibiotic resistant elements in regions with minimal human activities.

Moreover, antibiotics used for agricultural purposes, and other applications, for instance in aquaculture, enter the water bodies with runoffs. MPs as carrier of ARB and ARGs have potential to accumulate, transport and recirculate the antibiotic resistant elements into the aquaculture environment (Dong et al., 2021). Residual antibiotics might induce selective pressure and promote ARB and ARGs in the water environments. Antibiotics as a significant selection factor and a probable regulator of bacterial community in aquatic environment have been demonstrated by Mudryk (2002). The continuous movement of ARB and ARGs on MP surfaces across the environment indicate a potential health problem and broader ecological risks.

In urban water, plastics indicated accumulation, acquisition, antibiotic tolerance, and spread of antibiotic resistance (Yang et al., 2020). MPs in freshwater might play the role of a vector for antibiotic resistant elements. In aerobic sludge systems, Zhang et al. (2021) reported the potential spread of ARGs from the sludge to other environmental compartments, and Dai et al. (Dai et al., 2020) indicated that PVC MPs enhance the propagation of ARGs. Cheng et al. (2022) reported ARGs propagation by MPs in municipal wastewater. The studies indicate that MPs might aid in the spread of antibiotic resistance in freshwater bodies.

5.5. MPs compromise the removal efficacy of resistant elements

The ARGs removal efficiency in an aerobic sludge digestion system was deteriorated by the presence of MPs resulting in higher levels of ARGs in the digested sludge.

In a study, the removal efficiency of ARGs in a control aerobic sludge digestion system without exogenous MPs was compared with those containing MPs (PET, PVC and PE). They found the ARGs removal efficiency of the control reactor to be 59.6 - 96.7%. While the ARGs removal efficiencies in the presence of MPs were 32.1 - 91.2% (PVC), 28.7 - 93.8% (PE) and 46.0 - 92.5% (PET) (Zhang et al., 2021). The decrease in treatment efficacy of ARGs indicates an additional challenge posed by MPs in WWTPs.

MPs impacting biological processes for wastewater treatment might contribute in failure of WWTPs in removal of all antibiotic resistant elements. In a study on a pilot-scale upflow granular anaerobic sludge blanket (UASB) treating real wastewater, an addition of 50 PP MPs.gTS⁻¹ resulted in a 58% decrease in methanogenic activity. The accumulation of MPs in the sludge inhibited the anaerobic process (Pittura et al., 2021). Similar observations were made in other studies on anaerobic granular sludge system (Lin et al., 2020; Zhang et al., 2020). With the introduction of bisphenol A (BPA) in the system, the removal efficiency of chemical oxygen demand (COD) decreased by 30%. Polyether (PES) and BPA impacted the activities of protease, acetate kinase and coenzyme F_{420} . Furthermore, the bacterial diversity changed in the presence of PES and BPA (Lin et al., 2020). These changes might explain the inhibition of anaerobic activities. Therefore, MPs potentially impact the wastewater treatment performances in both aerobic and anaerobic systems.

In another study, granular PE and fibrous PA MPs compromised the disinfection efficacy of chlorine and UV disinfection. The decrease in disinfection efficiency was positively correlated with the increasing MP concentration. This was attributed to MPs reaction with disinfectants around them and MPs biofilm ability to provide microbial communities with protection from disinfectants (Shen et al., 2021). ARB, ARGs, and

MGEs are predicted to persist in MP biofilm and escape conventional water treatment techniques.

6. Conclusions and recommendations

This work presents a review conducted on research studies about the interaction of MPs and antibiotic resistant elements in freshwater ecosystems. The studies support the potential roles of MPs in selective enrichment of microbial species, and carriers of antibiotic resistant & mobile elements in the aquatic environment.

The coexistence of MPs with other pollutants in freshwater resources is predicted to amplify the associated health impacts. The MP microbial community is considered a new niche for potential pathogenic, antibiotic resistant species and genetic elements with potentially detrimental impacts on the aquatic ecosystem. The situation calls for improvement in treatment techniques and identification of MP pollution control strategies.

Further recommendations upon this review are:

- Research studies on MPs-antibiotic resistant element interactions are limited, and further studies targeting a wider range of aquatic environments are required to evaluate associated risks.
- The impact of MP pollution sources on the MP biofilm community remains understudied. The MPs-antibiotic resistant elements interaction traced across the water cycle could contribute to valuable information on pollution source tracking. Studies are required on tracking MP pollutions and MP biofilm composition from source to the marine environment through various possible pathways.
- Studies indicated that the characteristics of MPs impact the biofilm microbial community composition. Comparative studies on MP biofilm as a factor of MP characteristics in the aquatic environment might help design MP specific manufacturing, control, and removal strategies.
- There are no studies and analysis methods for nanoplastics (NPs) and biofilm formation on NP surfaces in water. The breakdown of MPs to NPs might provide increased surface area and binding-sites for the attachment of biological compounds in the water environment.
- Due to limited studies, the human exposure and additional risk associated with MP biofilm are not quantifiable across varying environmental conditions at this stage.

Declaration of Competing Interest

No conflict of interest exists in the submission of this manuscript.

Acknowledgments

The authors acknowledge the financial support of the European Union's Horizon 2020 research and innovation program in the frame of REWATERGY, Sustainable Reactor Engineering for Applications on the Water-Energy Nexus, MSCA-ITN-EID Project N. 812574, and the Spanish State Research Agency (AEI) and the Spanish Ministry of Science, Innovation and Universities through the project CALYPSOL-ATECWATER (RTI2018-097997-B-C33).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2022.100071.

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