



## Review

# Microplastic biofilms in water treatment systems: Fate and risks of pathogenic bacteria, antibiotic-resistant bacteria, and antibiotic resistance genes



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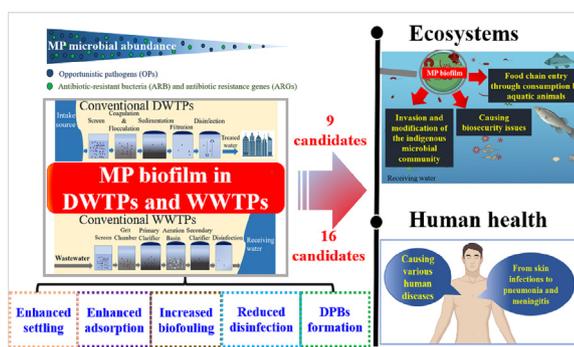
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## HIGHLIGHTS

- Pathogens, ARB, and ARGs in MP biofilm in DWTPs and WWTPs are reviewed.
- MPs retain mechanically and disinfection-resistant pathogens and ARB/ARGs.
- DWTPs retain nine potential pathogens and ARB/ARGs, and WWTPs retain sixteen.
- MP biofilm affects settling, adsorption, biofouling, disinfection, and DBP formation.
- Potential pathogens and ARB/ARGs can cause biosecurity and severe human illnesses.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Microplastics (MPs) biofilms in drinking water and wastewater treatment plants (DWTPs and WWTPs) have gained increasing attention due to their potential to come into close contact with humans. This review examines the fate of pathogenic bacteria, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in MP biofilms and their impacts on operations in DWTPs and WWTPs, as well as the associated microbial risks for ecology and human health. The literature shows that pathogenic bacteria, ARBs, and ARGs with high resistance can persist on MP surfaces and may escape treatment plants, contaminating drinking and receiving water. Nine potential pathogens, ARB, and ARGs can be retained in DWTPs and sixteen in WWTPs. While MP biofilms can improve the removal of MPs themselves, as well as the associated heavy metals and antibiotic compounds, they can also induce biofouling, hinder the effectiveness of chlorination and ozonation, and cause the formation of disinfection by-products. Furthermore, the operation-resistant pathogenic bacteria, ARB, and ARGs on MPs may have adverse impacts on receiving ecosystems, as well as human health, including a range of human diseases, from skin infections to pneumonia and meningitis. Given the significant implications of MP biofilms for aquatic ecosystems and human health, further research is necessary on the disinfection resistance of microbial populations in MP biofilm. This study provides valuable insights into the comprehensive understanding of the changes of MP biofilms in water and wastewater treatment systems as well as their impacts on ecology and human health.

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## 1. Introduction

Plastic production worldwide surpassed 360 million metric tons in 2018 and continues to rise annually (Amelia et al., 2021; He et al., 2022; Oberbeckmann et al., 2016). In aquatic environments, numerous microbial communities promote the development of biofilms on the surface of microplastics (MPs) (Nguyen et al., 2022). MP biofilms constitute a unique biological niche (He et al., 2022; Sturm et al., 2022) and are considered a reservoir for certain microorganisms, such as pathogenic bacteria, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) (Hu et al., 2021; Kelly et al., 2021; Kruglova et al., 2022; Mughini-Gras et al., 2021). Since biofilms quickly colonize MPs released into water environments (Tarafdar et al., 2021; Tarafdar et al., 2022), biofilms commonly coexist with MPs in water, making them a primary environmental concern related to MP pollution (Perveen et al., 2023). Several recent review articles have investigated the physicochemical characteristics, environmental consequences, infections, and antimicrobial resistance linked with MPs in natural environments (Debroy et al., 2022; He et al., 2022; Kaur et al., 2022; Stabnikova et al., 2022). However, a comprehensive understanding of MP biofilms in water and wastewater treatment systems remains incomplete.

MP contamination is gaining increasing attention in engineered water systems, such as drinking water treatment plants (DWTPs) and wastewater treatment plants (WWTPs), due to the potential for close contact with humans through probable sources of MP intake, including drinking water and receiving water from WWTPs. While DWTPs and WWTPs are designed to eliminate particles and pollutants, including MPs, these treatment plants are not completely effective at removing MPs, and the occurrence of MP in tap water and drinking water, including bottled water, has been frequently documented (Kirstein et al., 2021a; Pivokonsky et al., 2018). Moreover, WWTPs are often recognized as the primary source of MP discharge into urban waterways (Le et al., 2023; Magni et al., 2019). As a result, biofilms associated with MPs can also pass through a range of treatment steps. The fate of MP biofilms, including pathogens, ARB, and ARGs, can be influenced accordingly in these facilities. Unfortunately, studies on the potential impacts of operation procedures in DWTPs and WWTPs on the pathogens, ARB, and ARGs in MP biofilm are currently limited.

The effects of MP biofilm on the treatment efficiency of DWTPs and WWTPs, as well as ecology and human health, can be crucial if the MP load in the water treatment facility is escalating. MP biofilms can change the removal performance in the treatment systems. MP biofilm in water treatment facilities has been shown to improve the removal of MPs, as well as other pollutants such as heavy metals and organic compounds, by enhancing sedimentation and adsorption processes (Ahamed et al., 2020; Zhang et al., 2020b). However, the presence of MP biofilm in water treatment systems can also reduce sterilizing efficiency, as it can provide a protective layer for microorganisms and reduce the effectiveness of

disinfection processes (Shen et al., 2021a). In addition, pathogenic bacteria, ARB, and ARGs on MP biofilm can survive for a long time in the receiving ecosystem after WWTP discharge (Metcalfe et al., 2023). Thus, they can invade indigenous microbial communities and induce negative effects on the receiving habitat (Amaral-Zettler et al., 2020; Li et al., 2021). Moreover, ingesting these germs can lead to infections, antibiotic resistance, and other serious health issues in humans. Earlier studies have highlighted the impacts of MPs on wastewater treatment processes, with little attention paid to the effects of biofilm on MP (Mahon et al., 2017; Wu et al., 2021). Therefore, a more comprehensive understanding of the effects of MP biofilms on water and wastewater treatment systems, as well as their impacts on ecology and human health, is required.

This review aims to analyze the interactions between MP biofilm and drinking water and wastewater treatment procedures, with a particular focus on pathogenic bacteria, ARB, and ARGs. First, the abundance of MP in DWTPs and WWTPs was compiled. The characteristics of biofilms on MPs are subsequently discussed. Then, the study investigates the impact of DWTP and WWTP operations on these microorganisms in MP biofilms. The next part examines how MP biofilms affect the efficiency of water and wastewater treatment. The final part assesses the potential ecological and human health risks posed by pathogenic bacteria, ARBs, and ARGs that are resistant to treatment in MP biofilms. The ultimate goal of the review is to expand our understanding of MP contamination in DWTPs and WWTPs.

## 2. Microplastic abundance in drinking water and wastewater

MPs are found in various aquatic environments, including rivers and streams that serve as input sources of water for drinking water treatment facilities. Despite several barriers in conventional treatment processes in DWTP systems, a significant number of MPs smaller than 20  $\mu\text{m}$  can survive these processes, as reported in previous studies (Wu et al., 2022). In contrast, DWTPs can eliminate a great majority of MPs larger than 20  $\mu\text{m}$  (Bauerlein et al., 2022; Na et al., 2021). Specifically, the removal efficiency of small-sized MPs in traditional sand filtration is limited (Jung et al., 2022). For instance, Wu et al. (2022) reported that only 80.96 % of MPs of  $\leq 20 \mu\text{m}$  were removed in the DWTP of the Yangtze River region in China. Pivokonsky et al. (2018) also found that MPs with sizes ranging from 1 to 10  $\mu\text{m}$  could be present in treated water in DWTPs in the Czech Republic, with the number of MPs remaining as high as around 243–684 MPs/L. Therefore, DWTPs may still be vulnerable to the treatment of small-sized MPs, even with long-term operation of membrane filtering systems (Ding et al., 2021). It is also noteworthy from recent studies that a large piece of MPs can be fragmented into smaller sizes via UV oxidation and ozonation, which can be part of the treatment processes (Jung et al., 2022; Wang et al., 2020; Wu et al., 2022).

**Table 1**  
Overview of microplastic abundance in drinking water treatment plants in the previous studies.

Country	MP concentration	Study size range	Predominance			References
			Composition	Shape	Size	
Czech Republic	Raw water: 1383–4464 MPs/L Treated water: 243–684 MPs/L	≥ 0.2 μm	Raw water and treated water: PET, PP, and PE	Raw water and treated water: fragments, fibers, and spheres	Raw water and treated water: 1–10 μm	(Pivokonsky et al., 2018)
Germany	Raw water: 0–0.007 MPs/L in Drinking water: 0.0007 MPs/L	>20 μm	Raw water and drinking water: PE, PA, PEST, PVC, and epoxy resin	Raw water and drinking water: fragments	Raw water and drinking water: 50–150 μm	(Mintinig et al., 2019)
India	Raw water: 17.88 MPs/L - Pre-disinfection effluent: 17.53 MPs/L - Flocculation effluent: 17.11 MPs/L - Pulse clarification effluent: 6.99 MPs/L - Sand filtration effluent: 11.17 MPs/L Treated water: 2.75 MPs/L	≥ 0.7 μm	All the sampling stages: PET and PE	All the sampling stages: fibers and films/fragments	All the sampling stages: <100 μm	(Sarkar et al., 2021)
China	Raw water: 6614 ± 1132 MPs/L - Sedimentation effluent: 3141.65 ± 654.75 MPs/L (40.5–54.5 % raw water) - Sand filtration effluent: 1152.99 ± 342.11 MPs/L (29.0–44.4 % sedimentation effluent) - Ozonation: 1885.13 ± 138.60 MPs/L (55–72 % filtration effluent) - GAC filtration effluent: 1109.40 ± 54.65 MPs/L (56.8–60.9 % ozonation effluent) Final effluent: 930 ± 72 MPs/L	1–5000 μm	All the sampling stages: PET, PE, and PP	All the sampling stages: fibers, spheres, and fragments	All the sampling stages: 1–5 μm	(Wang et al., 2020)
Germany	Raw water (groundwater): 0.197 MPs/L Tap water: 0.006–0.074 MPs/L Drinking water: 0.001–0.102 MPs/L	≥ 5 μm	Raw water: PE and PP Tap water: PE and PET Treated water: PE and PA	N/A	All the samples: 5–20 μm	(Pittroff et al., 2021)
China	Raw water: 134.79 MPs/L - Coagulation and sedimentation effluent: 62.39 MPs/L (53.71 % removal efficiency) - Membrane filtration effluent: 67.80 MPs/L (– 8.67 % removal efficiency) Final effluent: 95.63 MPs/L Tap water: 13.23 MPs/L	≥ 0.7 μm	Raw water: nylon and PEST Final effluent: PEST, PVC, nylon, and PP Tap water: PEST, PS, and nylon	All the samples: fragments and fibers	Raw water: >200 μm Effluent: 100–200 μm Tap water: 100–200 μm	(Chu et al., 2022)
Brazil	Raw water: 330.2 MPs/L Treated water: 105.8 MPs/L	200–1000 μm	N/A	Raw water and treated water: fibers	N/A	(Ferraz et al., 2020)
China	Raw water: 2173–3998 MPs/L Treated water: 338–400 MPs/L Tap water: 267–404 MPs/L	≥ 1.0 μm	All water samples: PE, PP, and PET	All water samples: fibers and fragments	All water samples: 1–10 μm	(Shen et al., 2021b)
Indonesia	Water supply: 26.8–35.0 MPs/L - Aeration effluent: 14.8–18.4 MPs/L - Pre-sedimentation effluent: 9.0–11.7 MPs/L - Coagulation effluent: 7.5–15.6 MPs/L - Flocculation- Sedimentation effluent: 18.5–24.3 MPs/L - Filtration effluent: 13.8–29.7 MPs/L Treated water: 8.5–12.3 MPs/L	≥ 1.0 μm	Water supply: PE, PP, PS, and PET Treated water: PE, PP, and PET	All water samples: fibers and fragments	Water supply and treated water: 351–1000 μm	(Radityaningrum et al., 2021)
China	Raw water: 3738.36 ± 461.29 granular MPs/L and 2.8 fibrous MPs/L - Biological treatment effluent: 7789.1 granular MPs/L and 0.57 fibrous MPs/L - Coagulation & sedimentation effluent: 4478.5 granular MPs/L and 0.49 fibrous MPs/L - Ozonation & biological activated carbon (BAC) filtration effluent: 1837.3 granular MPs/L and 0.45 fibrous MPs/L - Micro-flocculation & sand filtration effluent: 1361.5 granular MPs/L and 0.08 fibrous MPs/L Treated water: 695.66 ± 39.32 granular MPs/L and 0 fibrous MPs/L	5 μm-5 mm for granular MPs 100 μm-5 mm for fibrous MPs	Granular MPs in raw water: PP, PE, PVC, and VINYON. Fibrous MPs in raw water: PET and VINYL Granular MPs in the treated water: PP, PVC, PE, and PA	All the samples: granules, fibers, and fragments	Granular MPs in raw water: 5–20 μm Fibrous MPs in raw water: 100–500 μm Granular MPs in the treated water: 5–20 μm	(Wu et al., 2022)
Spain	Water source: 0.96 ± 0.46 MP/L - Sand filtration effluent: 0.14 ± 0.05 MPs/L - GAC filtration effluent: 0.13 ± 0.06 MPs/L - Reverse osmosis effluent: 0.06 ± 0.04 MPs/L Finished water: 0.06 ± 0.04 MPs/L	20–5000 μm	Water source: PP and PEST Finished water: PEST and PP	All the water samples: fibers and fragments	MP fibers in the raw water: 200–500 μm MP fragments in the raw water: 500–1000 μm MP fibers in the finished water: 200–2000 μm MP fragments in the finished water: 200–500 μm	(Dalmau-Soler et al., 2021)
England and Wales (UK)	Raw water: 4.9 MPs/L Potable water: 0.00011 MPs/L	>25 μm	Raw water: PE, PET, and PP Potable water: PS and ABS	N/A	Raw water and potable water: <25 μm	(Johnson et al., 2020)
Thailand	Freshwater: 0.24–2.40 MPs/L	Freshwater:	Freshwater and treated	Freshwater and treated	Freshwater and	(Chanpiwat and

(continued on next page)

Table 1 (continued)

Country	MP concentration	Study size range	Predominance			References
			Composition	Shape	Size	
	Treated water: 0.24–1.00 MPs/L	≥ 50 μm	water: PP, PE, and PET	water: fibers and fragments	treated water: <300 μm	Damrongsiri, 2021)
China	Tap water: 0.3–1.6 MPs/L Water sources: 0.2–0.7 MPs/L	Treated water: >100 μm 10–5000 μm	Tap water: PET and rayon Water sources: PET and rayon	Tap water and water sources: fiber and fragment	Tap water and water sources: 500–1000 μm	(Zhang et al., 2020a)
German	Tap water: 0 MPs/L	≥ 10 μm	N/A	N/A	N/A	(Weber et al., 2021)
Sweden	Drinking water distribution system: 0.174 MPs/L	≥ 6.6 μm	PA, polyester, acrylic, PVC, PS, PE, PU, and PP	Fiber and fragment	<150 μm	(Kirstein et al., 2021b)
Mexico	Free drinking water fountains: 5 ± 2 to 91 ± 14 MPs/L	≥ 0.22 μm	PTT and epoxy resin	Fibers and fragments	<20 μm (32 %)	(Shruti et al., 2020)
China	Tap water: 0–1247 MPs/L	1–5000 μm	PE and PP	Fragments, fibers, and spheres	≤ 1000 μm	(Tong et al., 2020)
Denmark	Tap water: 0.31 ± 0.14 MP-like particles/L	≥ 100 μm	PET, PP, PS, and ABS	Fibers, fragments, films	N/A	(Feld et al., 2021)
Brazil	Tap water: 97 ± 55 to 219 ± 158 MPs/500 mL	0–5 mm	N/A	N/A	6–50 μm	(Pratesi et al., 2021)
Saudi Arabia	Bottled and tap water: 1.9–4.7 MPs/L	25–500 μm	PE, PS, and PET	N/A	N/A	(Almaiman et al., 2021)
Hong Kong	Tap water: 0.000–8.605 MPs/L	≥ 2.7 μm	N/A	Fibers and films	150–499 μm	(Lam et al., 2020)

N/A: not available.

Polyethylene terephthalate (PET); Polypropylene (PP); Polyethylene (PE); Polystyrene (PS); Polyamide (PA); Polytrimethylene terephthalate (PTT); Polyester (PEST); Acrylonitrile butadiene styrene (ABS); Polyvinyl chloride (PVC); Vinyl chloride-vinyl acetate copolymer (VINYL); Vinyon (VINYON).

The presence of MPs has been reported in both tap and bottled water. Initial research has shown the presence of MPs in raw and treated water from DWTPs in the Czech Republic (Pivokonsky et al., 2018). Subsequent investigations in several countries have detected MPs in tap and treated water (Table 1), with MP concentrations ranging from 0 to 1247 MPs/L (Johnson et al., 2020; Pittroff et al., 2021; Pratesi et al., 2021; Shruti et al., 2020; Tong et al., 2020). MPs have also been found in bottled water worldwide (Almaiman et al., 2021; Kankanige and Babel, 2020; Makhdoumi et al., 2021; Mason et al., 2018; Oßmann et al., 2018; Schymanski et al., 2018; Weisser et al., 2021; Wiesheu et al., 2016; Winkler et al., 2019; Zuccarello et al., 2019). The MP concentration in bottled water ranged from 1.4 and  $5.4 \times 10^7$  MPs/L (Kirstein et al., 2021a). MPs in potable water can originate from water sources, the water manufacturing process, and plastic-made packaging materials (Almaiman et al., 2021; Mason et al., 2018; Oßmann et al., 2018).

An overview of previous data of MPs in WWTPs reveals that a considerable number of MPs can travel through the treatment processes (see Table 2). WWTPs are designed to remove particulate contaminants from wastewater before discharging it into the receiving environment. As such, they can dramatically lower MPs concentrations from 1.86 to 31,400 MPs/L in influent (Franco et al., 2021; Hidayaturrahman and Lee, 2019; Le et al., 2023; Magni et al., 2019; Simon et al., 2018) to 0.004 to 340 MPs/L in effluent (Park et al., 2020; Van Do et al., 2022). However, because MP removal is not complete, ranging from 21.8 to 99.9 % (Magni et al., 2019; Park et al., 2020; Van Do et al., 2022), WWTPs are still regarded as a primary source of MP discharge into the aquatic environment. Magni et al. (2019) estimated that WWTPs in Italy could emit roughly 160 million MPs per day into the environment. Furthermore, Kim et al. (2022) observed MPs as small as 20 μm at a tertiary WWTP in South Korea for a year and estimated that the final effluent could yield a possible yearly load of 2900 million MP particles, equivalent to 0.54 kg, into rivers.

In general, a substantial number of small-sized MPs appear to overwhelm the fairly effective treatment efficiencies of DWTP and WWTP systems. As MP is always covered by a biofilm layer in the water environment (Li et al., 2022; Vaseashta et al., 2021), the biofilm associated with these MPs may have unique potential to pass through and be impacted by the processing of DWTPs and WWTPs. As a result, the following sections will describe the fate in microbial communities associated with MP biofilm in DWTPs and WWTPs.

### 3. Microplastic biofilms in the DWTPs and WWTPs

#### 3.1. Formation of MP biofilms

Biofilm formation is a dynamic process that includes microbial adherence, secretion of extracellular polymeric substances (EPS), and microbial multiplication (He et al., 2022; Tu et al., 2020). Due to their small size, hydrophobic rough surface, and extended half-life, MPs can easily become an effective substrate for colonization of microbes, resulting in a small ecological niche known as the “plastisphere” (He et al., 2022; Zettler et al., 2013). Once MPs enter aquatic systems, biofilm formation on them occurs rapidly (Leiser et al., 2020).

It is a common misconception that the low quantity of MPs and high-water quality in DWTPs would result in low biofilm content and limit investigations on microbial populations in MP biofilm. However, Pérez-Guevara et al. (2022) recently discovered organisms and biofilm growth on the surface of MPs in 63 drinking water samples collected from decentralized refill kiosks in the Mexico City metropolitan area, using scanning electron microscope (SEM) images. The SEM pictures revealed that diatoms and coccoid-shaped bacterial cells colonized the MPs, and bacteria with long filaments were also found. This finding suggests that there might be a substantial quantity of biofilm on the MP surface after the drinking water treatment system.

In wastewater, MP biofilm has been widely recognized in both WWTP processing operations (Kelly et al., 2021) and simulating experiments in the laboratory (Lai et al., 2022; Perveen et al., 2023). This is likely due to the presence of various nutrients in wastewater, which can stimulate the formation and development of biofilm on the MP surfaces (Nguyen et al., 2022; Parrish and Fahrenfeld, 2019). In addition to the indigenous microbial communities in inlet water, certain activated sludge (AS) free-living bacteria attached to MPs develop a unique bacterial composition of MP biofilms during secondary wastewater treatment (Kelly et al., 2021).

#### 3.2. Characteristics of MP biofilms

Previous studies have demonstrated that the plastisphere, which refers to microbial assemblages that differ from those found in surrounding environments, possesses distinct characteristics (Miao et al., 2019; Rummel et al., 2021). The composition of plastisphere species does not solely depend on the type of MP polymer (Di Pippo et al., 2020; Nguyen et al., 2022). Initial bacterial communities in MP biofilms show variation across

**Table 2**  
Overview of microplastic abundance in wastewater treatment plants in the previous studies.

Country	Information of WWTP	MP concentration	Study size range	Predominance			References
				Composition	Shape	Size	
South Korea	50 representative WWTPs nationwide	Influent: 10–470 MPs/L Effluent: 0.004–0.51 MPs/L	Influent: $\geq 45 \mu\text{m}$ Effluent: $\geq 100 \mu\text{m}$ $\geq 300 \mu\text{m}$	Influent and effluent: PP, PE, and PET	Influent and effluent: fragments and fibers	N/A	(Park et al., 2020)
New Zealand	Three WWTPs in Canterbury, New Zealand	Effluent: $1.3 \pm 0.6$ MPs/L	$\geq 10 \mu\text{m}$	PEST, PE, and PP	Fragments	N/A	(Ruffell et al., 2021)
Canada	WWTP in Saskatoon	Effluent: 1.76 MPs/L	$\geq 10 \mu\text{m}$	PE	Fibers	N/A	(Prajapati et al., 2021)
China	Urban WWTP in Hainan	Influent: $219.50 \pm 77.75$ MPs/L - Post-fine screen effluent: $111.53 \pm 65.68$ MPs/L - Post-secondary sedimentation tank effluent: $35.64 \pm 42.41$ MPs/L - Disinfection tank effluent: $74.62 \pm 36.76$ MPs/L Final effluent: $12.98 \pm 14.58$ MPs/L	$\geq 25 \mu\text{m}$	Final effluent: PET, PP, nylon, PE, and PS	Final effluent: fibers, fragments, and films	Final effluent: 25–380 $\mu\text{m}$	(Bao et al., 2022)
Daegu, South Korea	Three full-scale WWTPs in Daegu	Influent: 4200–31,400 MPs/L - Primary settling tank effluent: 1568–12,580 MPs/L - Secondary settling tank effluent: 433–7863 MPs/L - Coagulation effluent: 164–1444 MPs/L Final effluent: 33–297 MPs/L	$\geq 10 \mu\text{m}$	N/A	All the samples: microbead and fragments	N/A	(Hidayaturrahman and Lee, 2019)
China	Seven secondary WWTPs in Xiamen	Influent: 1.57–13.69 MPs/L Effluent: 0.20–1.73 MPs/L	$\geq 43 \mu\text{m}$	Influent and effluent: PP, PE, PS, and PET	Influent and effluent: granules, fragments, fibers, and pellet	Influent: 63–125 $\mu\text{m}$ Effluent: $\geq 125 \mu\text{m}$	(Long et al., 2019)
Italy	WWTP in northern Italy, the most anthropogenic region in Europe	In: $495 \pm 61$ MPs/L Pre-disinfection: $5.7 \pm 2.0$ MPs/L Post-disinfection: $5.8 \pm 2.7$ MPs/L	$\geq 25 \mu\text{m}$	All the samples: PE and PP	All the samples: fragments, films, and fibers	All the samples: 100–499 $\mu\text{m}$	(Galafassi et al., 2021)
Denmark	10 of the biggest WWTPs	Raw wastewater: 7216 MPs/L Treated wastewater: 54 MPs/L	10–500 $\mu\text{m}$	Raw wastewater: acrylate and PP Treated wastewater: PEST, PE, and acrylates	Raw wastewater and treated wastewater: particles and fibers	Raw wastewater: $\leq 100 \mu\text{m}$ Treated wastewater: $\leq 91 \mu\text{m}$	(Simon et al., 2018)
Scotland	Glasgow's big secondary WWTP on the Clyde	Influent: $15.70 \pm 5.23$ MPs/L - Grit and grease effluent: $8.70 \pm 1.56$ MPs/L - Primary effluent: $3.40 \pm 0.28$ MPs/L Final effluent: $0.25 \pm 0.04$ MPs/L	$\geq 65 \mu\text{m}$	Influent: alkyds, polystyrene-acrylic, PEST, polyurethane, and acrylic Final effluent: polyester, polyamide, polypropylene, acrylic, alkyd, PE, PS, and PET	All water samples: flakes, fibers, film, beads, and foam	All water samples: average $598 \pm 89 \mu\text{m}$	(Murphy et al., 2016)
Germany	12 WWTPs	Effluent: 0–0.05 MPs/L MP $>500 \mu\text{m}$ and 0.01–9 MPs/L MP $<500 \mu\text{m}$	$\geq 10 \mu\text{m}$	MP $> 500 \mu\text{m}$ : PE and PP MP $< 500 \mu\text{m}$ : PE, PVAL, PA, and PS	Both sample fractions: fibers	MP $>500 \mu\text{m}$ : 500–7200 $\mu\text{m}$ MP $<500 \mu\text{m}$ : 50–100 $\mu\text{m}$	(Mintenig et al., 2017)
Viet Nam	Three WWTPs in Da Nang	Influent: 183–443 MPs/L Effluent: 138–340 MPs/L	$\geq 1.6 \mu\text{m}$	Influent and effluent: PET, PE, and PVC	Influent and effluent: fiber and fragment	Influent and effluent: 1.6–5000 $\mu\text{m}$	(Van Do et al., 2022)
Iran	Ahvaz's biggest WWTP	Influent: 9.2 MPs/L - Primary sedimentation effluent: 4.7 MPs/L - Secondary sedimentation effluent: 1.36 MPs/L Final effluent: 0.84 MPs/L	$\geq 25 \mu\text{m}$	N/A	All the samples: fiber, film, and granule	Influent: 125–420 $\mu\text{m}$ - Primary sedimentation effluent: 125–420 $\mu\text{m}$ - Secondary sedimentation effluent: 25–125 $\mu\text{m}$ Effluent: 25–125 $\mu\text{m}$	(Takdastan et al., 2021)
Turkey	Secondary WWTP in Istanbul	Influent: 73.1 MPs/L - Physical treatment effluent: 30.7–34.3 MPs/L - Biological treatment effluent: 203 MPs/L Final effluent: 5.1–11.2 MPs/L	$\geq 25 \mu\text{m}$	Influent: PC and PUR Final effluent: PC, PUR foam, and PES	All the samples: fiber and fragment	All the samples: 500–1000 $\mu\text{m}$ and $>2000 \mu\text{m}$	(Vardar et al., 2021)
Spain	Industrial and urban WWTPs	Influent: 645.03–1567.49 MPs/L Effluent: 16.40–131.35 MPs/L	$\geq 100 \mu\text{m}$	Influent and effluent: PVC, PE, EAA, and HDPE	Influent and effluent: fibers, fragments, and flakes	Influent and effluent: 100–355 $\mu\text{m}$	(Franco et al., 2021)
Thailand	A conventional WWTP	Influent: $77 \pm 7.21$ MPs/L - After the grit trap: $57.33 \pm 8.08$ MPs/L - After the aeration tank: $96.67 \pm 30.09$ MPs/L - Effluent from the final clarifier: $10.67 \pm 3.51$ MPs/L - Effluent from ultrafiltration: $2.33 \pm 1.53$ MPs/L	$\geq 50 \mu\text{m}$	All the samples: PET, PE, and PP	All the samples: fibers	All the samples: 50–500 $\mu\text{m}$	(Tadsuwan and Babel, 2022)

(continued on next page)

Table 2 (continued)

Country	Information of WWTP	MP concentration	Study size range	Predominance			References
				Composition	Shape	Size	
Viet Nam	Four domestic WWTPs	Influent: 1.86–125.0 MPs/L Effluent: 0.14–0.813 MPs/L	>200 $\mu\text{m}$	N/A	Influent and effluent: fibers	Influent and effluent: fiber length 549–1248 $\mu\text{m}$ All the samples: 100–500 $\mu\text{m}$	(Le et al., 2023)
Italy	One of the largest WWTP	Influent: 2.5 $\pm$ 0.3 MPs/L - After the settler: 0.9 $\pm$ 0.3 MPs/L Effluent: 0.4 $\pm$ 0.13 MPs/L	$\geq$ 10 $\mu\text{m}$	Influent: acrylonitrile-butadiene, PE, and ethylene-propylene - After the settler: PEST, PE, PU, PA, and PP Effluent: PEST, PA, and PE	Influent: films, fragments, and lines - After the settler: films, fragments, and lines Effluent: lines, films, and fragments		(Magni et al., 2019)

N/A: not available.

High density polyethylene (HDPE); Polyvinyl alcohol (PVAL); Polycarbonate (PC); Polyurethane (PUR); Polyethersulfone (PES); Ethylene acrylic acid (EAA); Polyurethane (PU).

polymers; however, subsequent MP degradation processes in aquatic settings diminish these differences within plastisphere communities (Di Pippo et al., 2020; Pinto et al., 2019). Contrarily, the size of the plastic particle influences MP biofilm formation. Biofilm were found to colonize more easily on MPs with a small particle size of 75  $\mu\text{m}$  compared to larger MPs measuring 4000  $\mu\text{m}$  (Wu et al., 2022). Biofilm thickness in lake water and wastewater treatment systems fluctuates between several tens to hundreds of nanometers (Feng et al., 2023; Hossain et al., 2019). The biofilm on MPs accounted for approximately 194–311 mg dry mass/g MP in freshwater and 168–357 mg dry mass/g MP in wastewater (Pořizka et al., 2023). Moreover, biofilm on MPs contained  $10^7$ – $10^9$  cells or 16S rRNA gene copies/g MP, as determined by Flow Cytometry (FCM) or Polymerase Chain Reaction (PCR) techniques (Bydalek et al., 2023; Wu et al., 2019). Moreover, biofilms can enhance the potential of MPs to absorb contaminants such as persistent organic pollutants, heavy metals, and antibiotics (Kaur et al., 2022; Qiongjie et al., 2022; Sathicq et al., 2021). Additionally, MPs are often seen as potential vectors of harmful bacteria (Kaur et al., 2022; McCormick et al., 2016; Wu et al., 2019), ARB, and ARGs (Hu et al., 2021; Junaid et al., 2022; Mughini-Gras et al., 2021). These characteristics are believed to be persistent in MP biofilms in both DWTPs and WWTPs.

MP biofilms exhibit distinct properties compared to non-plastic biofilms in aquatic environments such as rivers and lakes. Due to their hydrophobic surface, buoyancy, and long transport distance, MPs provide a novel habitat for the selection and spread of unique microbial assemblages (McCormick et al., 2016). MP bacterial assemblages are less diverse and taxon-rich than those on other surfaces (Miao et al., 2019). Previous studies have reported higher levels of biofilm metabolic pathways for cofactors, amino acids, and vitamins on MPs than on natural substrates such as wood and cobblestone (Miao et al., 2019). Additionally, MP biofilms contain specific bacterial taxa such as *Pseudomonas*, which can degrade plastic polymers (McCormick et al., 2016). Furthermore, Wu et al. (2019) found distinct ARG profiles in MP biofilms, with an enrichment of multidrug resistance genes (e.g., *smeE*, *mdsC*) and antibiotic resistance genes (e.g., *qnrVC6*, *ermF*) compared to rock and leaf biofilms.

Numerous analytical techniques, encompassing visual analysis and molecular methodologies, have been employed to investigate the biofilm associated with MPs (Kaur et al., 2022). Visual analysis methods encompass SEM, Confocal Laser Scanning Microscopy (CLSM), and Atomic Force Microscopy (AFM). SEM analysis is used to investigate the morphology of MP biofilms (Nguyen et al., 2022), while CLSM is a commonly used method for quantifying the thickness of EPS (Hossain et al., 2019). Moreover, AFM can determine the morphological growth stages of biofilm on the MP (Tarafdar et al., 2021). Advanced molecular approaches involve the use of FCM, Fluorescence In Situ Hybridization (FISH), PCR, and Next-Generation Sequencing (NGS) techniques. Through the autofluorescence and optical scattering characteristics of FCM combined with visual stochastic network embedding (viSNE) (Sgier et al., 2016), biofilm characterization at the single-cell level is achievable. FISH and PCR facilitate the

detection of specific gene sequences as well as the identification of bacterial species present in biofilms (Debroj et al., 2022). NGS methods are extensively utilized for microbial identification, sequencing, and other focused tasks, which include sequencing coding/non-coding entities (Kaur et al., 2022). Furthermore, the evaluation of MP-associated biofilms has recently been conducted using infrared (IR) spectral characteristics (Battulga et al., 2022). To elucidate the structural characteristics of the biofilms formed on plastic surfaces, the researchers employed a spectral subtraction technique using the spectra of peroxide-treated and untreated PS-MP particles.

Biofilm can affect the extraction and identification of MPs. The biofilm that forms can alter the characteristics of MPs, such as size and density, thereby complicating MP extractions that rely on size separation (e.g., sieving and filtration) or density separation (Cashman et al., 2020; Rummel et al., 2017). Biofilms may camouflage MP particles, thus hindering their optical identification (Pořizka et al., 2023). Furthermore, surficial biofilms can potentially interfere with surface scanning in spectroscopic MP identification (Cashman et al., 2020). Therefore, it is recommend to remove biofilms prior to MP identification.

A recent study has highlighted the influence of environmental factors in adjacent waters on the structure of the plastisphere (Nguyen et al., 2022). Variables such as organic content, salinity, and the level of dissolved oxygen concentration in the water have been identified as significant determinants of plastisphere community patterns, impacting the order: organic content > salinity > DO concentration. Therefore, anthropogenically derived organic matter, predominantly found in wastewater and comprised of diverse molecules and fluorophores (Meng et al., 2013; Song et al., 2023) may also impact MP biofilms. Notably, pathogenic bacteria were specifically identified in the MP biofilm cultivated in water with high organic content (Nguyen et al., 2022). This implies that the communities of pathogenic bacteria, ARB, and ARGs in the plastispheres of DWTPs and WWTPs with a high amount of organic compounds can be distinct. Therefore, studying MP biofilm in drinking water and wastewater is necessary. Additionally, determining the microbiological alterations of pathogenic bacteria, ARB, and ARGs on MPs before and after treatment stages is essential to properly assess the efficacy of water treatment systems that may contain MPs.

### 3.3. Effects of DWTP operations on pathogenic bacteria, antibiotic resistance bacteria, and antibiotic resistance genes in MP biofilm

Whereas the occurrence of MP biofilms has been reported in DWTPs (Pivokonsky et al., 2018; Pratesi et al., 2021), there is a lack of studies investigating changes in microbial communities on MPs during the treatment process in DWTPs. Thus, this section aims to synthesize existing data on microbial communities in intake water sources of DWTPs (Hu et al., 2021; Mughini-Gras et al., 2021) and MP biofilm communities in laboratory-replicated drinking water studies (Chen et al., 2023, 2022, 2021).

The microbial communities on MPs in DWTPs are diverse and include opportunistic pathogens, ARBs, and ARGs. Hu et al. (2021) investigated MP contamination at a watershed level in Jiangxi, China, which serves as a key source of drinking water. The study found 58 human pathogenic microorganisms on MPs, with *Streptococcus mitis*, *Pseudomonas fluorescens*, *Pseudomonas savastanoi*, *Klebsiella pneumoniae*, *Pseudomonas entomophila*, *Pseudomonas protegens*, *Pseudomonas stutzeri*, *Salmonella enterica*, and *Aeromonas hydrophila* being the top ten species. Among the human pathogenic bacterial species enriched on MPs, *Pseudomonas stutzeri* and *Pseudomonas protegens* were most prevalent. *Streptococcus mitis* was also found to be a possible host of macrolide resistance genes on MPs. The study also found that mobile genetic elements (*intI1*) on MPs were crucial in the horizontal transfer of sulfonamide antibiotic resistance genes. Another study by Mughini-Gras et al. (2021) sampled MPs and their associated microbial communities from the Dutch part of the Rhine River near a drinking water abstraction point and found that taxa harboring potential pathogens (*Pseudomonas*, *Acinetobacter*, and *Arcobacter*) were concentrated in particular types of MPs, and that other risk-conferring signatures, such as the *sul1* and *erm(B)* antimicrobial resistance genes, were widespread. Both studies highlighted the selective enrichment of opportunistic infections on MPs and the need to pay special attention to pathogen mobility. It is important to note that the pathogens and ARGs discovered on MPs may pass through DWTPs.

Some microbes within the plastisphere's community exhibit resistance to DWTP treatment. To the best of our knowledge, three investigations have been conducted on MP biofilm and plastisphere in drinking water, which utilized laboratory simulations (Chen et al., 2023, 2022, 2021). Using a modeling system, Chen et al. (2023) explored the impact of tap water hydraulic conditions (0–2 m/s) on MP biofilm growth and microbial community composition. Their study found that opportunistic pathogens (OPs) associated with MPs, including *Curvibacter*, *Flavobacterium*, and *Sediminibacterium*, were more sensitive to hydraulic conditions and decreased with flow velocity. Conversely, the species of *Chryseobacterium*, *Microbacterium*, and *Burkholderia* increased with flow velocity, suggesting that these OPs were capable of adapting to harsh hydraulic conditions and remaining firmly attached to the plastisphere. The findings also demonstrate that certain microorganisms strongly adhered to MP surfaces and may withstand mechanical operations in DWTPs.

In another study, the MP biofilm in drinking water was investigated under various disinfection conditions (0, 0.5, 1.0, and 1.5 mg Cl<sub>2</sub>/L) (Chen et al., 2022). Although the disinfection duration was long (14 days), the heterotrophic plate count in the MP biofilm was still detected, ranging from  $7.73 \times 10^4$  CFU/cm<sup>2</sup> to  $3.91 \times 10^6$  CFU/cm<sup>2</sup>. Several OPs remained on the MPs. For example, the percentage of *Bacilli* (of the *Firmicutes*) in water samples increased with chlorine concentration. *Pseudomonas* was consistently present in all the MP biofilms. When the chlorine content exceeded a certain level (i.e., 1.5 mg/L), *Pseudomonas* became the dominant chlorine-resistant organisms. The findings suggest that several pathogenic bacteria present on MP biofilm may be resistant to disinfection in DWTPs.

In addition to serving as hydrophobic surfaces for microbial adhesion, MPs also function as carbon sources, affecting microbial activities in stagnant water, which is common in drinking water systems, particularly in water tanks and distributed small-scale drinking water purifiers (Chen et al., 2021). However, it should be noted that the three studies mentioned above used commercially available MPs that were carefully sterilized before being incubated in drinking water, and microbial growth may have been lower than that of MPs that harbor biofilm in actual DWTPs. Nevertheless, based on the aforementioned research, it can be concluded that certain bacteria may still exhibit resistance to treatment processes in DWTPs, such as mechanical actions and disinfection.

It is evident that MP biofilm can be highly impacted by other DWTP processes, such as coagulation-flocculation-sedimentation and filtration. However, as of March 2023, there are currently no reports on this subject. Earlier research that reported on microbial communities in bulk water found that the impact of coagulation-flocculation and sedimentation on microbial communities was minimal, with no evident shift in bacterial populations occurring as a result of these processes (Lin et al., 2014; Poitelon

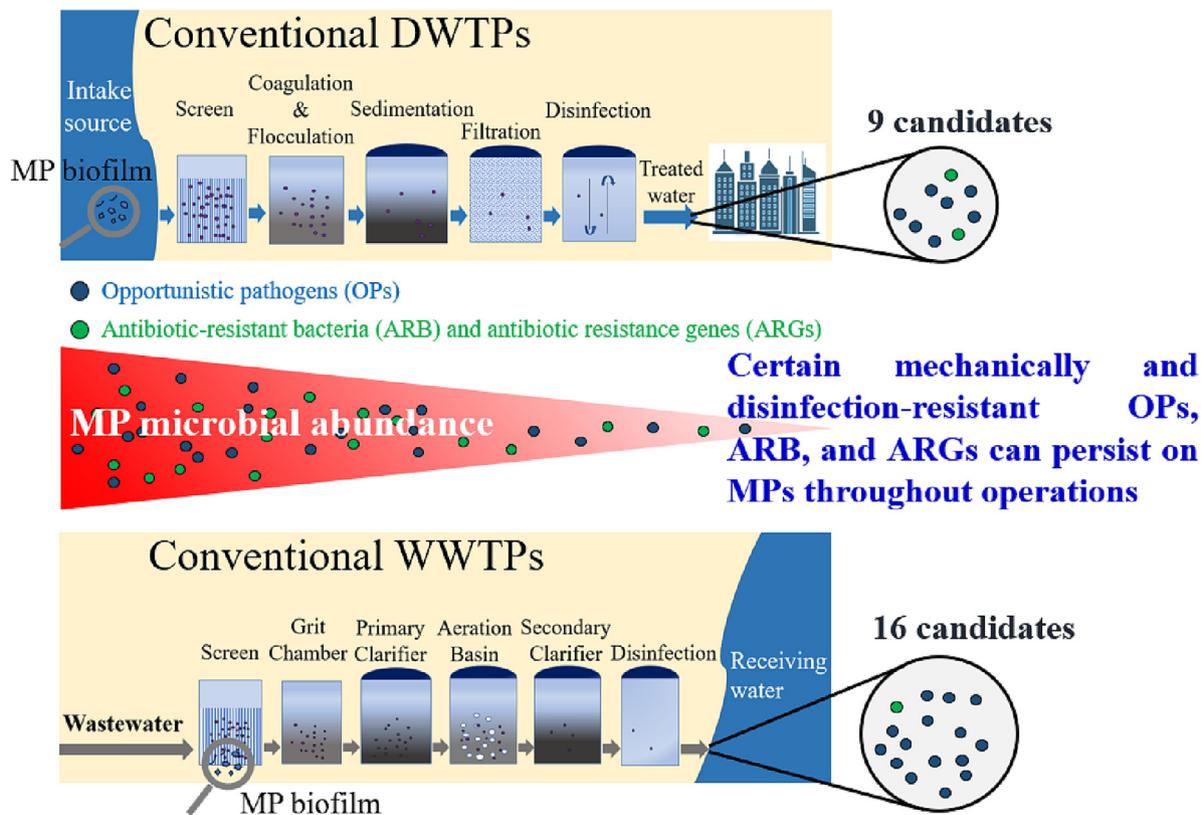
et al., 2010). However, filtration, such as sand or granular activated carbon (GAC) filtration, did have an impact on the microbial community composition of water (Lin et al., 2014; Vignola et al., 2018). These findings suggest that biofilm bound to MPs can be greatly influenced by these processes.

In summary, since MPs are not entirely removed in DWTPs, biofilms on MPs can be transported from the intake source water and pass through the treatment processes. MP bacterial groups in raw water can be classified as sensitive or resistant based on treatment effects. Fig. 1 illustrates changes in the abundance of pathogenic bacteria, ARB, and ARGs on MP in DWTPs. Although their abundance on MPs can be efficiently reduced by water treatment processes, nine resistant microorganisms, including taxa harboring potential pathogens (*Pseudomonas*, *Acinetobacter*, *Arcobacter*, *Chryseobacterium*, *Microbacterium*, *Burkholderia*), and ARGs (*sul1*, *erm(B)*), are likely to survive on MPs throughout the operations of DWTPs (Fig. 1). Considering the scarcity of data regarding MP biofilms in DWTPs, laboratory-based studies on MP biofilms in water sources and drinking water may provide valuable insights into the changes within plastisphere communities in DWTPs. In the future, it will be essential to identify which process within the treatment plant most significantly impacts biofilms. This knowledge will facilitate effective management strategies for MP-associated pathogenic bacteria, ARB, and ARG in DWTPs.

#### 3.4. Effects of WWTP operations on pathogenic bacteria, antibiotic resistance bacteria, and antibiotic resistance genes in MP biofilm

There have been only a few studies on MP biofilm in WWTPs, including those by (Galafassi et al., 2021; Kelly et al., 2021; Kruglova et al., 2022). Among these, Kelly et al. (2021) conducted the first examination of bacterial communities attached to MPs in two WWTPs, demonstrating the diversity of microbial assemblages in MP biofilm across the WWTPs. The authors observed that distinct microbial assemblages colonized MP particles from all samples, which were retained to the plastic surfaces and not flushed off during MP collection. Many potentially pathogenic bacterial taxa, including the family *Campylobacteraceae*, the genus *Arcobacter*, and the genus *Aeromonas*, were found in lower abundance on MPs than on influent sewage MPs, indicating that MP transit through WWTPs operated to lower these species. In addition, other taxa related to human diseases, such as the genus *Acinetobacter* and its family *Moraxellaceae*, were commonly found on MPs in both influent and effluent wastewater, suggesting that taxonomic abundance was unaffected by the wastewater treatment. Furthermore, sequences from several bacterial genes, including those of the *Enterobacteriaceae* family, the *Pseudomonas* genus, the *Sphingomonas* genus, and the *Sphingomonadaceae* family, were significantly more abundant on MPs in the treatment processes than in sewage, indicating that these taxa increased in abundance during wastewater. Overall, these findings suggest that if wastewater treatment fails to reduce the number of various bacteria, including potentially harmful bacterial taxa, on MPs, they may be released into the environment.

Disinfection is a critical process in the WWTP system that effectively removes harmful germs from wastewater before it is released into the environment. As a result, sterilization is expected to efficiently eliminate microorganisms in MP biofilm, including potentially harmful bacteria and ARGs. To test this hypothesis, Galafassi et al. (2021) examined the community composition (via 16S rRNA gene amplicon sequencing) and the concentration of antibiotic resistance genes (via qPCR) of biofilm on MPs and planktonic bacteria in treated (pre- and post-disinfection) wastewaters in a WWTP in northern Italy, an area with the highest anthropogenic impact in Europe. In the MP biofilm, ten potentially harmful bacteria were identified. Surprisingly, the authors found that the disinfection procedure by ozonation had no effect on the composition of the plastisphere's bacterial community, as well as the abundances of ARGs and MRGs on MPs. The findings suggest that MPs can serve as carriers of potentially harmful bacteria (e.g., *Chryseobacterium* abundant in the plastisphere) and ARGs (e.g., *sul2*, a common resistance gene against sulfonamides) in WWTPs despite the disinfection process, particularly based on ozonation.



**Fig. 1.** The changes in microplastic-associated pathogens, antibiotic-resistant bacteria (ARB), and antibiotic-resistant genes (ARGs) in drinking water and wastewater treatment plants (DWTPs and WWTPs) under operational impacts. Nine potential pathogenic bacteria and ARB/ARGs in the DWTPs were identified in DWTPs, including *Pseudomonas*, *Acinetobacter*, *Arcobacter*, *Chryseobacterium*, *Microbacterium*, *Burkholderia*, *Lactobacillus*, *sul1*, and *erm(B)* ARGs. Sixteen pathogenic bacteria and ARB/ARGs were found in WWTPs, including *Pseudomonas*, *Acinetobacter*, *Arcobacter*, *Campylobacteraceae*, *Aeromonas*, *Enterobacteriaceae*, *Sphingomonas*, *Sphingomonadaceae* family, *Chryseobacterium*, *Leptotrichiaceae*, *Streptococcus*, *Dietzia*, *Neisseriaceae*, *Lactobacillus*, *Streptococcus*, and *Sul2* ARGs. The information was retrieved from several recent studies (Hu et al., 2021) (Mughini-Gras et al., 2021) (Chen et al., 2022) (Chen et al., 2023; Galafassi et al., 2021) (Kelly et al., 2021; Kruglova et al., 2022).

Kruglova et al. (2022) reported consistent results indicating that core potentially pathogenic bacteria and ARGs in MP biofilms were less altered by treatments in WWTPs. The authors assessed the bacterial populations of MP-associated bacteria from several phases of municipal wastewater treatment processes in Finland (influent, after pre-treatment, after activated sludge (AS), and final treatment). After all treatment stages, the core bacterial groups remained attached to MPs and were not removed by the wastewater treatment process. Numerous harmful bacteria, including *Leptotrichiaceae*, *Streptococcus*, *Dietzia*, and *Pseudomonas* species, were found in MP samples from all treatment phases. Additionally, dangerous bacteria, such as the *Neisseriaceae* family, were not observed in the influent but occurred in the MP biofilm. This is because some of the AS free-living bacteria attached to MPs generate a unique bacterial composition of MP biofilms following subsequent treatment. Furthermore, antibiotic-resistant organisms, such as *Streptococcus*, *Pseudomonas*, and *Lactobacillus*, were found in MP biofilms. Perveen et al. (2023) and Martínez-Campos et al. (2021) cultured MPs in collected wastewater effluent from WWTPs in Spain. The authors concluded that MPs in treated wastewater might act as a reservoir for ARGs over time. In general, the majority of pathogenic bacteria, ARB, and ARGs on MPs are efficiently decreased through treatment procedures in WWTPs; nevertheless, some mechanically and disinfection-resistant OPs and ARGs can escape through the treatments. Sixteen pathogenic bacteria, ARB, and ARGs were summarized as the ones that can survive on MPs during procedures (*Pseudomonas*, *Acinetobacter*, *Arcobacter*, *Campylobacteraceae*, *Aeromonas*, *Enterobacteriaceae*, *Sphingomonas*, *Sphingomonadaceae* family, *Chryseobacterium*, *Leptotrichiaceae*, *Streptococcus*, *Dietzia*, *Neisseriaceae*, *Lactobacillus*, *Streptococcus*, and *Sul2*). A graph depicting the changes in the abundance of pathogenic bacteria, ARBs, and ARGs on MPs in WWTPs was present (Fig. 1). Since the conditions during the WWTP

process do not effectively reduce the variety of clinically relevant bacteria associated with MPs, it is conceivable that MPs may serve as protective reservoirs for potentially pathogenic bacteria and ARGs within these systems. This could increase the risk to both ecological and human health upon exposure. Furthermore, just as in the case of DWTPs, comprehensive information regarding MP biofilms in each process within the WWTPs will be required in the future. Such information is crucial for determining which procedures have the most significant influence on biofilm presence within the treatment plant.

Overall, biofilms associated with MPs from WWTPs and DWTPs have been found to be substantial sources of infections, ARB, and ARGs, and may have major impacts on the ecological and human health. Moreover, these biofilms tend to persist throughout the treatment processes. The quantity of infections, ARB, and ARGs found in MP biofilms in WWTPs (sixteen) is approximately double that found in DWTPs (nine). This result suggests that infections, ARB, and ARGs in MP biofilms can negatively affect the performance of water and wastewater treatment systems. The effects of MP biofilms on treatment efficiency are described in the following section.

#### 4. Potential effects of MP biofilm on the performance of DWTPs and WWTPs

Biofilms can significantly alter the properties of MPs, such as their shape, size, and density, leading to an increase in their sedimentation rate. Firstly, the EPS matrix of biofilm can cause MPs to become adhesive, leading to increased aggregation and sinking rates. Secondly, fouling organisms may increase both the size and the density of the particles, reducing their buoyancy and facilitating deposition (Lee et al., 2022). Zhang et al.

(2020b) demonstrated that MP biofilm improved the removal effectiveness of coagulation/flocculation coupled with sedimentation (CFS) treatment, increasing the removal rate from 0.3 % to 16.5 % under normal operating circumstances in typical water treatment plants. Moreover, MP biofilm is considered a metabolic hotspot and an essential region for the decomposition of dissolved organic carbon (DOC) (Peter et al., 2011). Various bacteria residing in MP biofilm may stimulate the development of microaggregates for organic matter co-degradation (Zhang and Chen, 2020). MP biofilm comprises numerous bacteria, including those that can biodegrade plastic polymers (Nguyen et al., 2022), thereby aiding in plastic biodegradation.

Biofilm on MPs can improve their adsorption capacity for heavy metals, such as copper, cadmium, zinc, lead (Pb), and antibiotics like norfloxacin (Liu et al., 2022; Luo et al., 2022; Qiongjie et al., 2022). Ahamed et al. (2020) observed that the presence of biofilm on low-density polyethylene (LDPE) surfaces considerably enhanced Pb adsorption, resulting in a 13-fold higher equilibrium adsorption capacity (1602 g/m<sup>2</sup>) compared to when biofilm was absent (124 g/m<sup>2</sup>). He et al. (2023) reported that the development of biofilms increased norfloxacin adsorption by 50.6 %, 24.2 %, and 46.0 % on polyvinyl chloride, polyamide, and high-density polyethylene MPs, respectively. Since MP biofilm promotes MP sedimentation in WWTPs sludge (Lee et al., 2022), such toxic compounds adsorbed on MP surfaces may be more readily eliminated from bulk water in treatment facilities compared to MPs without biofilm. In this context, the removal of MPs from DWTPs and WWTPs can have the additional benefit of removing associated pollutants. Although the enhanced adsorption capacity of MPs by biofilm may lead to more accumulation of hazardous substances in the plastsphere, this also requires additional attention when recycling the WWTP sludge for soil fertilization. Moreover, the incomplete removal of MPs in DWTPs and WWTPs may ultimately result in hazardous compounds in the MP biofilm remaining in the treated water or being released to the receiving water source.

MP biofilm has the potential to impact membrane treatment systems through biofouling. Biofouling refers to the deposition of microorganisms on membrane surfaces, followed by the development of a biofilm. A previous study found that biofouling caused by bacteria and EPS in feed water is a primary cause of ultrafiltration membrane fouling (Yu et al., 2016). Microbes associated with MP biofilm can act as initial attachments on the membrane surface, resulting in biofilm succession. Hence, microorganisms and EPS linked with MP biofilm might exacerbate the biofouling phenomena in membrane-based water/wastewater treatment.

Moreover, MP biofilm can impair disinfection efficacy in DWTPs and WWTPs. Biofilms on MPs have been found to be more disinfection-resistant than planktonic bacteria (Boni et al., 2021; Hou et al., 2021). Shen et al. (2021a) found that MPs served as a significant protective canopy for bacteria during water/wastewater disinfection using ultraviolet and chlorine. By interacting with disinfectants, MPs can reduce the concentration of disinfectants surrounding them, safeguarding bacteria enriched on the surface of MPs (Shen et al., 2021a). MP biofilms may also allow wastewater microorganisms, such as *Escherichia coli*, to circumvent WWTP treatments. Thus, water and wastewater containing biofilm-coated MPs may require modifications to disinfection protocols, such as disinfectant dose and reaction time, to ensure effective disinfection.

MP biofilm has been found to cause the formation of disinfection by-products (DBPs) in treated water. A biofilm consists various types of microorganisms and EPS, which are rich in organic compounds. These compounds can be transformed into toxic DBPs, such as *N*-nitrosodimethylamine (NDMA) and dichloroacetonitrile (DCAN), during disinfection treatment. In a previous study, it was demonstrated that DBPs were generated during the chloramination of drinking water biologically active carbon media, with each gram of media producing 0.80 ± 0.27 ng NDMA and 18.7 ± 3.3 ng DCAN (Di Tommaso et al., 2019). Additionally, Xiong et al. (2022) found that MPs increased the production of DBP precursors from microbial by-products. However, the potential formation of DBPs via MP biofilm is not yet fully understood. Given the significant cytotoxicity and genotoxicity of various DBPs, it is imperative to seriously consider the formation potential of these by-products from

organic compounds related to MP biofilm. Overall, five potential effects of MP biofilm on the performance of water and wastewater treatment systems are summarized, including enhanced MP removal, enhanced adsorption for heavy metals and antibiotic compounds, enhanced biofouling, reduced the effectiveness of disinfection, and DBPs formation (Fig. 2). Understanding these impacts can assist in the operation of treatment systems.

## 5. Potential ecological risks of operation-resistant pathogenic bacteria, antibiotic resistance bacteria, and antibiotic resistance genes on MPs

In aquatic environments, MP biofilm can protect plastsphere bacteria, enabling them to survive for extended periods in receiving ecosystems. Researchers evaluated the survivability of human pathogens attached to MPs through mesocosm incubation studies, simulating MP migration downstream in the river-estuary-marine-beach continuum after WWTP discharge (Metcalf et al., 2023). The author discovered that pathogens remained viable for at least 25 days, indicating that potential pathogens, ARB, and ARGs in MP biofilms can spread widely into receiving water sources while being transported over long distances across aquatic environments. Furthermore, the distinct microbial population in MP biofilm from WWTPs can exchange genes with indigenous bacteria of the receiving waters via horizontal and vertical gene transfer pathways (Arias-Andres et al., 2018). Therefore, microbial invasion and altered microbial ecology in the receiving habitat can be considered the initial effects of MP biofilm (Li et al., 2021).

Secondly, the potential pathogens, ARBs, and ARGs on the surface of MPs during WWTPs can pose a danger to the biosecurity of receiving habitats and even human water security (Amaral-Zettler et al., 2020; Li et al., 2021; Zettler et al., 2013). Lastly, researchers have reported that various aquatic animals, including invertebrates and fish, may ingest MPs as food particles (Lusher et al., 2017; Okamoto et al., 2022; Phillips and Bonner, 2015; Silva-Cavalcanti et al., 2017). As a result, the infections, ARB, and ARGs connected with MPs can enter the food chain in aquatic environments and eventually be consumed by humans through the consumption of contaminated food (Dong et al., 2021; Foley et al., 2018). The ecological impacts of potential pathogens, ARBs, and ARGs in MP biofilm discharged from WWTPs are summarized in Fig. 3.

MPs accumulate in the sludge from WWTPs that is used as biofertilizer in agricultural fields (Corradini et al., 2019; Rolsky et al., 2020), raising concerns about the potential impacts of MP biofilms on soil environments. However, research on MP biofilms in soil environments is still lacking (Ya et al., 2021). Currently, no information is available on the roles of MP microorganisms in WWTP sludge-amended soil. Since MP-associated

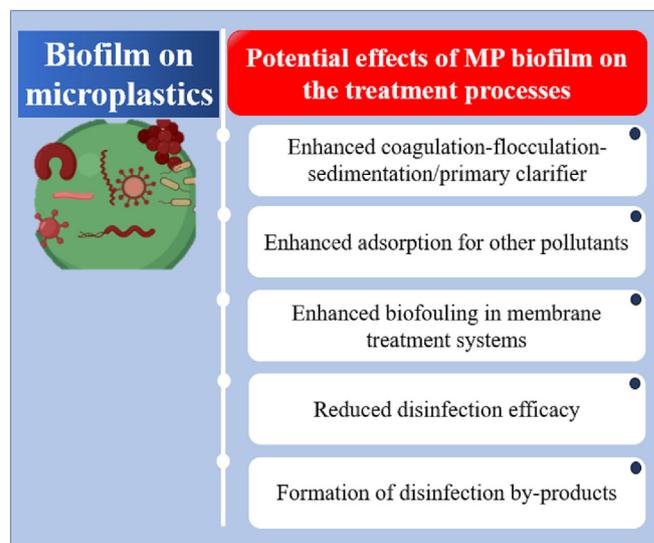


Fig. 2. Potential effects of microplastic biofilm on the working performance of drinking water and wastewater treatment processes.

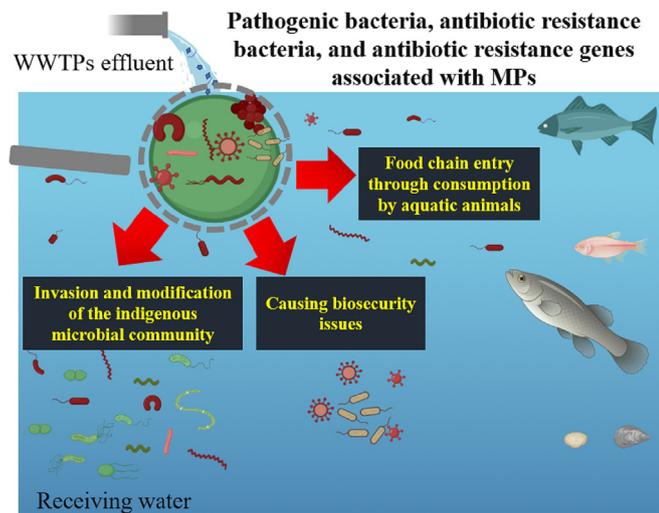


Fig. 3. Potential ecological effects of pathogenic bacteria, antibiotic resistance bacteria, and antibiotic resistance genes associated with MPs in the receiving water. MP biofilm microbes are represented by microbes in red. Microbes in green are indigenous microbes.

pathogens, ARB, and ARGs tend to be resistant to WWTP treatment processes, some may be retained in sludge after treatment and ultimately affect soil ecology. In the context of this hypothesis, MP biofilms in WWTP sludge may pose microbiological concerns in farming soil. The enrichment of pathogens and ARGs in pasture and vegetables may have adverse effect on food safety (Zhu et al., 2022), potentially raising human health hazards.

## 6. Potential human health risks of operation-resistant pathogenic bacteria, antibiotic resistance bacteria, and antibiotic resistance genes on MPs

Both pathogenic microorganisms and ARGs can have serious consequences for human health. Pathogens are intruders that attack host

organisms and can thrive in the warm and moist environments provided by human bodies. Emerging infectious illnesses caused by various organisms have long been a source of concern and continue to be the leading cause of death worldwide, especially in developing countries (Sarmah et al., 2018). Additionally, antibiotic resistance can lead to increased virulence, pathogenicity, disease outbreaks, and transmission, resulting in increased illness, hospitalization, and even mortality (Ashbolt Nicholas et al., 2013; Berendonk et al., 2015).

There are at least three mechanisms by which antibiotic resistance in drinking water can endanger human health: (1) humans can be directly infected by antibiotic-resistant pathogens after drinking contaminated water, but no human-to-human transmission occurs; (2) sustained human-to-human transmission occurs after direct infection with a pathogenic or opportunistic ARB; and (3) environmental ARGs in drinking water can transfer into human pathogens (Chang et al., 2015).

The pathogens, ARB, and ARGs linked to MPs that can pass through DWTPs and WWTPs (Fig. 1) may pose various health risks to humans (Table 3). Several microbes that cause skin disorders, such as the genera *Pseudomonas* and *Dietzia* (Koerner et al., 2009; Mena and Gerba, 2009), can also cause diarrhea, such as the family *Enterobacteriaceae* and the genus *Aeromonas* (Moxley, 2022; von Graevenitz and Mensch, 1968). Certain microbes can cause extremely deadly infections, such as *Acinetobacter*, which can cause pneumonia and meningitis (Dijkshoorn et al., 2007; Visca et al., 2011), and the *Campylobacteraceae* family, which can cause bacteraemia and septicaemia (Collado et al., 2013; Shange et al., 2020), while the *Neisseriaceae* family is responsible for bacterial meningitis, septicaemia, and gonorrhoea (Wong et al., 2015).

Moreover, ARGs linked with MPs such as *sul1* and *sul2*, tend to confer resistance to the effects of sulfonamide antibiotics, which are widely used in human medicine, animal production, and aquaculture to treat bacterial, protozoal, and fungal diseases (Braschi et al., 2010; Xie et al., 2022). *Erm(B)* antimicrobial resistance genes are resistant to macrolide antibiotics, which are used to treat human infectious illnesses such as community-acquired bacterial pneumonia and gonorrhoea (Babić et al., 2017; Xie et al., 2022).

In conclusion, bacteria attached to MPs in both drinking water and wastewater can have substantial impacts on human health. Thus, further

Table 3

Potential health risks from microplastic biofilm-associated pathogens, antibiotic-resistant bacteria (ARB), and antibiotic-resistant genes (ARGs) in water and wastewater treatments.

Potential pathogens/ARB/ARGs	Possible risks on human health	References
Genus <i>Pseudomonas</i>	Eye and skin infections, life-threatening illnesses in burn, surgery, and immunocompromised patients	(Mena and Gerba, 2009)
<i>Acinetobacter</i>	Pneumonia, skin- and soft-tissue infections, wound infections, urinary tract infections, meningitis, and bloodstream infections	(Dijkshoorn et al., 2007; Visca et al., 2011)
Arcobacter	Human enteric diseases and extraintestinal diseases, resistance to antimicrobials, and large rate of multidrug resistance	(Ferreira et al., 2016; Lehner et al., 2005)
Family <i>Campylobacteraceae</i>	Gastroenteritis in humans and more severe illnesses such as bacteremia and septicemia	(Collado et al., 2013; Shange et al., 2020)
Genus <i>Aeromonas</i>	Diarrheal disease, cellulitis, and septicemia	(von Graevenitz and Mensch, 1968)
Family <i>Enterobacteriaceae</i>	Diarrhea in all animal species including human beings	(Moxley, 2022)
Genus <i>Sphingomonas</i>	The control of <i>Sphingomonas</i> can prevent thymoma	(Higuchi et al., 2021)
<i>Chryseobacterium</i>	Bacteremia/sepsis, ventilator-associated pneumonia, indwelling device-associated infection, urinary tract infections, peritonitis, shunt infections, surgical and burn wound infections	(Bhalla et al., 2018; Sudharani and Saxena, 2011)
<i>Leptotrichiaceae</i>	Mucositis, oral lesions, wounds, and abscesses	(Eribe and Olsen, 2017)
<i>Streptococcus</i>	Scarlet fever, rheumatic heart disease, glomerulonephritis, and pneumococcal pneumonia	(Patterson, 1996)
<i>Dietzia</i>	Human skin infections	(Koerner et al., 2009)
<i>Neisseriaceae</i> family	Bacterial meningitis and septicaemia, and gonorrhoea	(Wong et al., 2015)
<i>Lactobacillus</i>	<i>Lactobacilli</i> can invade the human gastrointestinal and urinary systems and cause illness	(Slover and Danziger, 2008)
<i>Microbacterium</i>	Endophthalmitis, hematogenous, mitral valve endocarditis, and sepsis	(Funke et al., 1997)
<i>Sphingomonadaceae</i> family	Member of <i>Sphingomonadaceae</i> family ( <i>Sphingomonas paucimobilis</i> ) can cause of bloodstream infections with a significant risk of death	(Laupland et al., 2022; Lin et al., 2010)
<i>Streptococcus mitis</i>	<i>Multidrug-resistant Streptococcus mitis</i> possess macrolide and tetracycline resistance genes.	(Pimenta et al., 2019; Poutanen Susan et al., 1999)
<i>Sul1</i> and <i>sul2</i> ARGs	Sulfonamide-resistant gene	(Xie et al., 2022)
<i>Erm(B)</i> ARGs	Macrolide-resistant gene	(Xie et al., 2022)

investigations on the microbial communities associated with MPs and their removal effectiveness in DWTPs and WWTPs are necessary.

## 7. Conclusions

Small-sized MPs (<20 µm) in intake source water and influent wastewater can persist in DWTPs and WWTPs, respectively, due to incomplete removal of MPs during treatment processes. Consequently, MP biofilm, which constantly exists with MPs in the aquatic environment, can also be present in the treated water. Under operations of DWTPs and WWTPs, certain mechanically and disinfection-resistant OPs, ARB, and ARGs can persist on MPs. In DWTPs, nine potential pathogens, ARBs, and ARGs can be retained, while WWTPs can retain sixteen. MP biofilm has several effects on DWTP and WWTP processing efficiency, including increased removals of MPs due to higher settling capacity, and enhanced removals of heavy metals and organic compounds due to better absorptive ability than bare MPs. However, due to the incomplete removal of MPs in DWTPs and WWTPs, MP biofilm becomes a hotspot for pollutants. Additionally, MP biofilm can increase biofouling in membrane treatment systems, reduce the efficacy of chlorination and ozonation disinfection, and cause the formation of DBPs. The operation-resistant pathogenic bacteria, ARB, and ARGs on MP biofilm can cause adverse alterations to receiving ecology, as well as major human illnesses. Nevertheless, there is currently a lack of data on harmful bacteria and ARGs associated with MPs in drinking water and treated wastewater. Future research should focus on quantifying and characterizing harmful microorganisms and ARGs on MPs and their fate in DWTPs and WWTPs. Most importantly, it is crucial to investigate the disinfectant resistance of microorganisms in MP biofilm.

## Data availability

Data will be made available on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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