Chapter 9
The Microplastic-Antibiotic Resistance Connection

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Abstract Microplastic pollution is a big and rapidly growing environmental problem. Although the direct effects of microplastic pollution are increasingly studied, the indirect effects are hardly investigated, especially in the context of spreading of disease and antibiotic resistance genes, posing an apparent hazard for human health. Microplastic particles provide a hydrophobic surface that provides substrate for attachment of microorganisms and readily supports formation of microbial biofilms. Pathogenic bacteria such as fish pathogens *Aeromonas* spp., *Vibrio* spp., and opportunistic human pathogens like *Escherichia coli* are present in these biofilms. Moreover, some of these pathogens are shown to be multidrug resistant. The presence of microplastics is known to enhance horizontal gene transfer in bacteria and thus, may contribute to dissemination of antibiotic resistance. Microplastics can also adsorb toxic chemicals like antibiotics and heavy metals, which are known to select for antibiotic resistance. Microplastics may, thus, serve as vectors for transport of pathogens and antibiotic resistance genes in the aquatic environment. In this book chapter, we provide background information on microplastic biofouling ("plastisphere concept"), discuss the relationship between microplastic and antibiotic resistance, and identify knowledge gaps and directions for future research.

9.1 Introduction

Microplastic (<5 mm, GESAMP 2019) pollution is a widespread and global environmental problem that is projected to increase in upcoming decades creating significant challenges for its management and prevention (Borrelle et al. 2020; Jambeck

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et al. 2015). Transport of microplastic from land via headwater streams and large rivers to the ocean (Hurley et al. 2018; Jambeck et al. 2015; van Wijnen et al. 2019) is an important component of the microplastic pollution cycle, and plastic particles can now be found globally throughout all ecosystem components including the atmosphere, terrestrial landscapes, aquatic freshwater and marine environments, and all types of biota including seafood species commonly consumed by humans (Bank and Hansson 2019).

Microplastics represent a novel substrate for marine bacteria including both fish and human pathogens (Dang and Lovell 2016; McCormick et al. 2014; Zettler et al. 2013) and are also a reservoir for metal resistance and antibiotic resistance genes. The role of microplastics in the spread of antibiotic resistance is a relatively new research topic that has garnered significant interest by scientists (Bank et al. 2020; Bowley et al. 2021; Guo et al. 2020; Parthasarathy et al. 2019; Radisic et al. 2020). The indirect effects of microplastics have not been well studied especially in the context of seafood safety and global food security, and these effects may pose a significant hazard for human health regarding the spread of disease (Bank et al. 2020; Guo et al. 2020). The specific objectives of this chapter were to (1) provide background information on microplastic biofouling and describe the concept of the "plastisphere" (Zettler et al. 2013), (2) discuss the relationship of microplastic and antibiotic resistance, and (3) identify knowledge gaps and directions for future research.

9.2 The Plastisphere Concept

One of the critical mechanisms of the microplastic antibiotic resistance connection is the "plastisphere" concept. This concept was originally presented in the seminal paper by Zettler et al. (2013) who reported that microbial communities attached to plastic debris were diverse and composed of heterotrophs, autotrophs, predators, and symbionts and were distinct from the surrounding marine waters. These plastic particle surfaces represented a novel substrate and/or ecological habitat within the water column and on the surface of the open ocean (Amaral-Zettler et al. 2015, 2020; Bowley et al. 2021; Oberbeckmann et al. 2018; Wright et al. 2020; Zettler et al. 2013). Microplastic particles have hydrophobic surfaces, with no net charge, upon entering the ocean as virgin artificial materials; however, they can quickly become colonized by microbial biofilms (Bowley et al. 2021; Wright et al. 2020; Zettler et al. 2013). The development of this concept was important for forming scientific questions regarding the overall direct and indirect impacts of microplastic pollution primarily because of the long residence time of microplastic in the environment and the potential for long-range transport and the associated risks of transfer of pathogens and disease (Bowley et al. 2021). Pathogenic microbes such as *Vibrio* spp. have been reported in high abundance within the plastisphere (Amaral-Zettler et al. 2020; Bowley et al. 2021; Zettler et al. 2013; Zhang et al. 2020) and although not all *vibrios* are pathogenic, they often prefer lower salinity found in coastal and estuarine areas, thus highlighting the importance of the plastisphere regarding its distribution, abundance, fate, and transport (Bowley et al. 2021; Thompson et al. 2004). These identified risks and the processes related to microplastic and microbe interactions are complex and are influenced by ocean currents (Hale et al. 2020), sources, fate and transport dynamics, trophic transfer and food web complexity, horizontal gene transfer and attachment properties (Arias-Andres et al. 2019), buoyancy and sinking properties of microplastics, variation, and uptake by farmed (Sun et al. 2020a) and wild seafood taxa, leading to subsequent human exposures (Bowley et al. 2021; Zhou et al. 2020).

9.3 Antibiotic Resistance

The introduction of antibiotics for the treatment of infectious disease is one of the most important advances in healthcare. The global spread of resistance in bacteria, particularly in human pathogens, presents major challenges for treatment and preventing the spread of infections (Ventola 2015). Annually, in the European Union/European Economic Area, an estimated more than 33,000 deaths and more than 800,000 cases of “impacted life-years” are attributable to infections caused by antibiotic-resistant pathogens, with direct and indirect estimated costs of more than 1.5 B€ (Cassini et al. 2019). The World Health Organization (WHO) has predicted the advent of infectious diseases for which no antibiotic treatment will be available (WHO 2019).

Antibiotic resistance is a natural phenomenon. Misuse and over use of antibiotics has led to the development, selection, and global spread of antibiotic resistance (Roberts and Zembower 2020). Selection pressure from the presence of antibiotics or other antimicrobial compounds like heavy metals and biocides leads to the enrichment of antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) in bacteria from humans, animals, and the environment (Francino 2016; Gullberg et al. 2014; Marathe et al. 2013; Seiler and Berendonk 2012). Horizontal gene transfer (HGT) is a fundamental force driving bacterial evolution and contributes to the dissimilation of ARGs in both clinical and environmental bacteria (Boto 2010; Emamalipour et al. 2020; Jain et al. 2003). Antimicrobial compounds like antibiotics, biocides, and heavy metals can drive the development of antibiotic resistance and stimulate horizontal transfer of antibiotic resistance genes (Andersson and Hughes 2014; Jutkina et al. 2018; Zhang et al. 2018), thus aiding selection and dissemination of antibiotic resistance.

9.4 Microplastics and Antibiotic Resistance

Microorganisms attach themselves to surfaces forming a complex matrix of biopolymers and microbial cells known as biofilm (Dang and Lovell 2016). Formation of biofilms protect bacteria from unfavorable conditions in the environment (Donlan 2002). Biofilms are ubiquitous in aquatic environments and play an important role in various biological and ecological processes (Guo et al. 2018). Aquatic biofilms serve as a sink for various contaminants, like heavy metals, and antibiotics that are known to select for antibiotic resistance and stimulate horizontal transfer of antibiotic resistance genes (Gullberg et al. 2014; Guo et al. 2018; Jutkina et al. 2018; Richard et al. 2019). Accordingly, antibiotic resistance genes have been detected in natural aquatic biofilms (Balcázar et al. 2015; Guo et al. 2018).

Microplastic particles provide a hydrophobic surface that readily supports formation of microbial biofilms, where environmental conditions are the main drivers of biofilm formation (Oberbeckmann et al. 2018; Rummel et al. 2017). Pathogenic bacteria such as fish pathogens *Aeromonas* spp., *Vibrio* spp., and opportunistic human pathogens like *E. coli* can invariably be present in these biofilms (Kirstein et al. 2016; Silva et al. 2019; Viršek et al. 2017). Microplastics can selectively enrich both antibiotics and antibiotic-resistant bacteria on their surfaces in landfill leachates, freshwater, as well as in sea water (Su et al. 2020; Sun et al. 2020b; Wang et al. 2020; Wu et al. 2019). Thus, microplastics may serve as a vector for transport of pathogens in the aquatic environment.

Several methods have been used for detecting and quantifying ARGs associated with marine plastics including selective isolation of resistant bacteria and phenotypic antibiotic sensitivity testing, whole genome sequencing, shotgun metagenomics, and quantitative polymerase chain reaction (qPCR). Culture-based methods involving isolation of bacteria on a culture media followed by antibiotic sensitivity testing is a traditional approach used for studying antibiotic resistance (Khan et al. 2019). Zhang et al. (2020) carried out isolation and characterization of antibiotic-resistant marine bacteria from microplastic particles collected from marine aquaculture sites using a combination of seven antibiotics and a non-selective media. They showed presence of several multidrug-resistant marine bacteria including pathogenic *Vibrio* species on microplastics (Zhang et al. 2020). In contrast, other studies carried out selective isolation of pathogens like *Vibrio* spp. (Laverty et al. 2020) and *E. coli* (Song et al. 2020) showing presence of multidrug-resistant pathogens on marine microplastics. Recently, a study reported whole genome sequences (WGS) of antibiotic-resistant fish pathogens isolated from marine plastics (Radisic et al. 2020). With the advent of next-generation sequencing technology, WGS analysis of pathogens has become common and affordable tool for genotyping and epidemiology in clinics (Quainoo et al. 2017). WGS analyses are effective in elucidating the total metabolic potential of microorganisms and understanding the genetic basis of antibiotic resistance (Grevskott et al. 2020; Hendriksen et al. 2019). Although this is true, WGS data on microplastic-associated bacteria is largely lacking.

Only a small proportion of bacteria present in an environment can be cultivated in the lab. This limits detection and quantification of antibiotic resistance genes present in uncultivable bacteria using traditional methods (Lloyd et al. 2018; Stewart 2012). Methods like qPCR analysis or shotgun metagenomics, that use the total genomic DNA extracted from a given sample, partly overcome this limitation. Using qPCR, Wang et al. (2020) showed enrichment of ARGs like *sul1*, *tet*A, *tet*C, *tet*X, and *ermE* on plastic particles in both freshwater and sea water (Wang et al. 2020), while another study showed selective enrichment of *strB*, bla_{TEM}, *ermB*, *tet*M, and *tet*Q on microplastic particles in landfill leachates (Shi et al. 2020). These studies selected a limited number of ARGs for their analysis. In contrast, using recently developed high-throughput qPCR screening that can analyze more than 200 ARGs, Lu et al. (2020) showed presence of between 34 and 43 different ARGs on the surface of microplastic particles collected from vegetable soil (Lu et al. 2020).

Shotgun metagenomics gives an overview of the total bacteria and associated genes present in a given sample (Simon and Daniel 2011). Using this method Yang et al. (2019) found a total of 64 ARG subtypes that provide resistance against 13 different classes of antibiotics on macroplastics and microplastics collected from the North Pacific Gyre. Along with enrichment of ARGs, the study also found enrichment of metal resistance genes on microplastics (Yang et al. 2019). This study and several of the earlier described studies show presence of clinically important ARGs, like *sul1*, *tet*A, *tet*C, *tet*X, *ermE*, *aac(3)*, *macB*, and bla_{TEM}, that are invariably found in human pathogens, on microplastic particles (Alcock et al. 2020), thus suggesting that microplastics in the environment act as reservoirs for clinically important antibiotic resistance genes.

Microplastics originate from a variety of processes and invariably ends up in the marine environment via streams and large rivers (Hurley et al. 2018; Jambeck et al. 2015). High levels of microplastics reach the wastewater treatment plants (WWTP) (Dris et al. 2015). Although most of the microplastics are removed during primary and secondary waste treatment, smaller microplastics may still be present in the treated effluents (Talvitie et al. 2017). Treated effluents have low concentrations of microplastic particles but the high volume of effluents released may leads to considerable contamination of the aquatic ecosystem (Murphy et al. 2016; Talvitie et al. 2017). WWTPs receive municipal and/or hospital waste which invariably contains both human pathogens and clinically important antibiotic resistance genes (Le et al. 2016; Marathe et al. 2017, 2018, 2019; Rizzo et al. 2013). Treated effluents from waste water treatment plants are recognized as one of the major sources of environmental pollution with antibiotic resistance genes and resistant pathogens (Alexander et al. 2020; Czekalski et al. 2014; Karkman et al. 2019). The presence of microplastic particles in waste water effluents, thus, presents opportunities for antibiotic-resistant pathogens to colonize and form biofilms on plastic particles. This may lead to further dissemination of resistance in the marine environment via microplastics. Although this is true, there is limited knowledge on the impact of microplastics from treated effluents from WWTP on dissemination of ARGs in the aquatic environment.

Microplastic particles adsorb several chemicals like antibiotics, biocides, and heavy metals (Chen et al. 2020; Godoy et al. 2019; Mammo et al. 2020; Wang et al. 2020). The presence of antibiotics and active metabolites from such agents in the environment leads to selection of multidrug resistance among both clinical and environmental bacteria. Similarly, biocides and heavy metals like copper and mercury are known to co-select for antibiotic resistance (Francino 2016; Gullberg et al. 2011, 2014; Imran et al. 2019; Marathe et al. 2013; Seiler and Berendonk 2012). Adsorption of these chemicals on plastic surfaces containing microbial biofilm may lead to selection pressure in the plastisphere, resulting in active selection of antibiotic resistance on microplastic surfaces. In accordance, Imran et al. (2019) has concluded that co-contamination with microplastics and heavy metals results in development and spread of multiple drug-resistant human pathogens through co-selection mechanisms (Imran et al. 2019). Studies have shown that very low levels of antibiotics and biocides not only can select for antibiotic resistance but also can induce horizontal transfer of ARGs (Gullberg et al. 2011; Jutkina et al. 2018; Zhang et al. 2018). Moreover, bacteria in biofilms are more efficient in horizontal gene transfer compared to planktonic bacteria (Abe et al. 2020). Accordingly, studies have shown increased horizontal gene transfer in presence of microplastics via conjugation (Arias-Andres et al. 2018, 2019). Although extensive research on selection of resistance and promotion of horizontal gene transfer by antimicrobial compounds has been carried out, there is limited knowledge on the effect of adsorbed chemicals on plastisphere bacteria, especially, with reference to selection and transfer of antibiotic resistance genes on microplastic particles.

9.5 Conclusions and Directions for Future Research

Microplastics are emerging pollutants that have been detected in a range of environments. With the current trend of plastic consumption and its global production, the environmental pollution and related environmental effects caused by microplastics are expected to increase (Borrelle et al. 2020). Microplastics provide surfaces for the microorganisms to form biofilms (plastisphere) (Zettler et al. 2013). The processes and mechanisms involved in biofilm formation on microplastics are largely unclear. In-depth studies on deciphering the succession of microbes and understanding the effect of different factors that may influence biofilm formation on microplastic particles, such as the environmental conditions and the age of microplastic particles are needed (Su et al. 2020; Yang et al. 2020). Moreover, there are a limited number of studies reporting WGS of bacteria associated with microplastics (Li et al. 2019; Radisic et al. 2021). Bacteria associated with microplastics may play different ecological roles and could also be useful for bioremediation (Debroas et al. 2017). Hence, understanding the metabolic potential of bacteria in plastisphere using WGS is necessary.

Studies have investigated the composition of biofilms on microplastics and shown presence of both fish and human pathogens as well as clinically important antibiotic resistance genes (Dong et al. 2021). However, the risks associated with presence of pathogens in terms of human or fish exposure and the ability of microplastic-associated pathogens for causing infections is not fully understood. In-depth risk assessment studies on the effect of pathogen carrying microplastics on fish and human health are thus warranted. Microplastics originating in different compartments like WWTPs or aquaculture sites may carry different microbiota. WWTPs and aquaculture sites usually have presence of both antibiotic-resistant pathogens and microplastics (Cabello et al. 2016; Rodriguez-Mozaz et al. 2015). There is invariably selection pressure due to presence of antibiotics or biocides along with presence of resistant bacteria in these sites (Cabello et al. 2016; Edo et al. 2020; Yang et al. 2014). These environments could play an important role in enrichment and dispersal of pathogenic bacteria and ARGs to the marine ecosystem via microplastics. Although microplastics have been shown to increase HGT (Arias-Andres et al. 2018, 2019), the impact of microplastics on evolution and dissemination of antibiotic resistance in pathogens and environmental bacteria is largely unknown. In order to understand the indirect effects of microplastics, the relationship and interactions between microplastics and ARGs, as well as the impact of their association on aquatic environment especially on marine environment and sea food safety, needs to be further assessed. Holistic multidisciplinary studies on fate, migration, and potential environmental risks posed by microplastics through dissemination and evolution of antibiotic resistance are needed in the future, for better understanding the indirect effects of microplastic pollution.

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