Review

Selection for antimicrobial resistance in the plastisphere

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HIGHLIGHTS

• The Plastisphere refers to microbial communities that colonise plastics.
• This review highlights three mechanisms by which AMR evolution may be driven in the Plastisphere:
  • Horizontal gene transfer of ARGs may increase in the Plastisphere.
  • AMR selective or co-selective compounds adsorb to microplastics.
  • AMR selective or co-selective chemicals are impregnated within the plastic matrix.

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ABSTRACT

Microplastics and antimicrobials are widespread contaminants that threaten global systems and frequently co-exist in the presence of human or animal pathogens. Whilst the impact of each of these contaminants has been studied in isolation, the influence of this co-occurrence in driving antimicrobial resistance (AMR) in microplastic-adhered microbial communities, known as ‘the Plastisphere’, is not well understood. This review proposes the mechanisms by which interactions between antimicrobials and microplastics may drive selection for AMR in the Plastisphere. These include: 1) increased rates of horizontal gene transfer in the Plastisphere compared with free-living counterparts and natural substrate controls due to the proximity of cells, co-occurrence of environmental microplastics with AMR selective compounds and the sequestering of extracellular antibiotic resistance genes in the biofilm matrix. 2) An elevated AMR selection pressure in the Plastisphere due to the adsorbing of AMR selective or co-selective compounds to microplastics at concentrations greater than those found in surrounding mediums and potentially those adsorbed to comparator particles. 3) AMR selection pressure may be further elevated in the Plastisphere due to the incorporation of antimicrobial or AMR co-

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Abbreviations: AMR: antimicrobial resistance; ARB: antimicrobial resistant bacteria; ARG(s): antimicrobial resistance gene(s); E. coli: Escherichia coli; HGT: horizontal gene transfer; MGE(s): mobile genetic element(s); QS: quorum sensing.

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1. Introduction

The increasing threat of anthropogenic stressors to global One Health systems (i.e., humans, animals and the environment (Robinson et al., 2016)) has resulted in the proposal of a new approach to sustainability involving planetary boundaries wherein human society can safely exist and develop (Rockström et al., 2009). One such boundary considers ‘novel entities’, where safe thresholds can be monitored in terms of the emissions of chemicals, including both antimicrobials and microplastics (Gwenzi, 2022). With over 120 trillion microplastic (sizes 0.1 μm–5 mm (Thompson et al., 2004)) particles estimated to be floating in the global ocean (Lindeque et al., 2020), microplastics are the most prevalent type of marine plastic pollution. Simultaneously, antimicrobials can accumulate in aquatic ecosystems (Hayes et al., 2022), owing to the widespread misuse and overuse of antibiotics. As a result, selection for antimicrobial resistance (AMR) has been documented within environmental settings, leading to the spread of AMR bacteria (ARB) and AMR genes (ARGs) in environments across the globe (Gillings and Stokes, 2012). AMR represents one of the greatest threats to human health, with an estimated 5 million deaths associated with bacterial AMR infections in 2019 alone (Murray et al., 2022).

In many cases, microplastics will co-occur in environments alongside antimicrobials, ARGs and ARB (Fig. 1). Previous research has primarily discussed these contaminants in isolation within singular environments, yet the combined effects of these co-contaminants likely exacerbates the threats they pose individually. A key concern resulting from the coexistence of these contaminants is the role of microplastics as novel ecological habitats, supporting the formation of polymicrobial biofilms. These microplastic-attached communities, referred to as ‘the Plastisphere’ (Zettler et al., 2013), have not only been found to be distinct from their surrounding environment and other, natural debris, but may also be platforms for the attachment of AMR bacteria and potential human or animal pathogens (Lear et al., 2022; Ormsby et al., 2023).

Still, the significance and nature of the unique properties of microplastics (Fig. 2) that shape AMR within the Plastisphere, or in supporting the colonisation of ARB, is not clear. Furthermore, the mechanisms which may drive the emergence of AMR in microplastic-associated communities due to their frequent co-occurrence with antimicrobials and other AMR selective pressures are yet to be understood. This review aims to discuss the potential roles of microplastics in the selection for AMR.
2. Plastisphere communities

Although there are many characteristics of microplastics that facilitate and influence the formation of the Plastisphere (Fig. 2), the importance of the microplastic substrate itself in shaping Plastisphere communities is unclear (see meta-analysis from Wright et al. (Wright et al., 2021)). For example, community composition has been found to differ according to plastic polymer (Frère et al., 2018; Hansen et al., 2021; Muthukrishnan et al., 2019; Vaksmaa et al., 2021), particle size (Sun et al., 2022a; Wang et al., 2022a; Wu et al., 2022a; Banerjee et al., 2022; Phothakwanpracha et al., 2021; Liu et al., 2022a; Kim et al., 2022; Wang et al., 2022b; Li et al., 2022a; Zha et al., 2022; Zhao et al., 2023) or even microplastics of different colours (Lear et al., 2022; Wen et al., 2020). On the other hand, previous research has found that abiotic factors, including temperature, nutrient availability, depth and salinity are largely responsible for Plastisphere community profiles (Wright et al., 2021; Amaral-Zettler et al., 2020; Wright et al., 2020; Oberbeckmann et al., 2018), rather than microplastic-dependent features.

One of the barriers thus far to Plastisphere research is the frequent omission of natural substrate controls to compare community diversity and abundance (see Metcalf et al. (Metcalf et al., 2021)). Several studies have recently confirmed that Plastisphere communities are distinct from free-living communities (Zettler et al., 2013; Wen et al., 2020; Oberbeckmann et al., 2018; Bryant et al., 2016; De Tender et al., 2015; Pinnell and Turner, 2019; Pinnell and Turner, 2020; Aguila-Torres et al., 2022; Deng et al., 2022; Yu et al., 2022; Li et al., 2022b; Oberbeckmann et al., 2014; Wang et al., 2020; Hoellein et al., 2017; Amaral-Zettler, 2022), but less commonly whether they are distinct from microbial communities attached to control substrates. So far, Plastisphere communities have been found to differ significantly from steel (Muthukrishnan et al., 2019; Lee et al., 2008; Rogers et al., 1994), the rhizosphere (Shi et al., 2022a), glass (Lee et al., 2008; Vosshage et al., 2018; Ogonowski et al., 2018; Woodall et al., 2018; Kirstein et al., 2018; Pinto et al., 2019; Kirstein et al., 2019), cobblestone (Miao et al., 2019), wood (Muthukrishnan et al., 2019; Oberbeckmann et al., 2018; Miao et al., 2019; Song et al., 2022; Kesy et al., 2019), seston (Hoellein et al., 2017; Kesy et al., 2019; McCormick et al., 2016; McCormick et al., 2014), picoplankton (Bryant et al., 2016), seaweed (Quilliam et al., 2014; Metcalf et al., 2022), sand (Delacuvelerie et al., 2019), gravel (Agostini et al., 2021), leaves (Wu et al., 2019), cellulose (Ogonowski et al., 2018; Tagg et al., 2019), quartzite (Wang et al., 2022c), fabric (Woodall et al., 2018), sponge and fish (Schmidt et al., 2014). In contrast, a core microbiome shared across microplastic and control substrates has been found to occur with glass (Oberbeckmann et al., 2014; Pinto et al., 2019; Parrish and Fahrenfeld, 2019; Richard et al., 2019; Kesy et al., 2016; Dang et al., 2008; Erni-Cassola et al., 2020), basalt (Kelly et al., 2022), wood (Song et al., 2020; Hu et al., 2021), ceramic (Pinnell and Turner, 2019), and stones (Naz et al., 2016). These comparisons are crucial in developing our understanding of the Plastisphere, as free-living controls can only provide evidence to support the role of surface attachment in selecting for unique, pathogenic or AMR communities, and do not highlight the importance of and risk posed by microplastics over natural substrates.

3. Microplastics and the selection of AMR

Regardless of whether the Plastisphere is distinct to communities occupying surrounding environments or neighbouring surfaces,
Microplastics possess greater persistence and dispersal capabilities than other materials due to their recalcitrant surface, ever-increasing volume, and incomparable transport abilities (Wright et al., 2021). Likewise, if microplastics do harbour unique communities, it could also be suggested that microplastics may select for particular traits within those communities, including AMR. Therefore, it is crucial to determine whether Plastisphere communities have the potential to enrich AMR and human or animal pathogens over natural material controls.

Our understanding of the evolution of AMR is informed largely by studies adopting clinically relevant concentrations of antimicrobials in vitro. However, recent research has uncovered that selection for AMR can occur at very low antibiotic concentrations, similar to those found in wastewater or even surface waters contaminated with antibiotic residues (Fig. 1) (Murray et al., 2018). One of the mechanisms driving the evolution of AMR in natural bacterial communities is horizontal gene transfer (HGT): the transmission of ARGs via mobile genetic elements (MGEs) due to conjugation, transformation or transduction between the same or even phylogenetically distinct species. Selection for de novo mutations, clonal expansion of resistant cells or HGT of ARGs typically increases under an elevated selective pressure, including the presence of selective (e.g., antibiotics) or co-selective (e.g., heavy metals) compounds. Co-selection is the indirect selection for AMR where either a single gene confers resistance to several compounds (cross-resistance) or where multiple ARGs are harboured on an MGE, such as a plasmid (co-resistance) (Murray et al., 2019).

AMR bacteria have been found to be selectively enriched on microplastics over free-living counterparts (Yang et al., 2019b), and metatranscriptomic evidence has recently identified that, not only were ARGs present in sampled polyvinyl chloride and polylactic acid Plastisphere communities, but they were actively expressed to a greater extent than planktonic water communities (Wu et al., 2022b). Additional interactions between microplastics and AMR have also been investigated, including the increased persistence of ARGs in microplastic biofilms (Yang et al., 2022) and, to a lesser extent, the role of microplastics as vectors of AMR within the food chain (additional summary of relevant literature can be found in Supplementary Material, Table 1). However, in recent years, researchers have begun to elucidate the importance of the Plastisphere in the selection for and emergence of AMR (Table 1).

Importantly, these potential mechanisms driving the selection for AMR within the Plastisphere (Table 1) have not yet been critically synthesised and the importance of microplastics in facilitating this at a greater extent than biofilms associated with natural controls is inconclusive, with much remaining to be understood. Here, we address possible reasons for why microplastics may promote both ecological (between taxa) and evolutionary (within taxa) selection of AMR.

We suggest three mechanisms by which microplastics may promote selection for AMR within the Plastisphere (Fig. 3):

1. Due to the proximity of cells in the Plastisphere, co-occurrence of environmental microplastics and selective or co-selective compounds, and the sequestering of extracellular ARGs in the biofilm matrix, rates of HGT are higher in the Plastisphere than in free-living counterparts.
2. Microplastics are known to adsorb both AMR selective or co-selective compounds at concentrations greater than those found in surrounding mediums, thus increasing selection or co-selection pressure for AMR in the Plastisphere if bioavailable.
3. During the manufacture of plastic polymers, AMR selective or co-selective compounds are often incorporated into the plastic matrix, leading to an increased selection or co-selection pressure for AMR local to the Plastisphere if bioavailable.

Though the literature used to support these hypotheses were not gathered in a fully systematic way, we have provided our literature search methodology, search terms and a detailed database of relevant literature in the Supplementary Material.

3.1. Horizontal gene transfer

Biofilms in general are associated with increased AMR due to the closer proximity of individual bacteria, promoting the HGT of MGEs bearing ARGs (Arias-Andres et al., 2018). The same is proposed for microplastic-associated biofilms, with biofilm-inducing quorum sensing (QS) systems facilitating cell-to-cell communication, the release of eDNA (Zhang et al., 2022b) and induction of type IV secretion systems (Amaral-Zettler et al., 2020; Kaur et al., 2021; Abe et al., 2020).

Using laboratory microcosm assays, Zhang et al. (Zhang et al., 2022a) reported that the conjugal antibiotic-resistance plasmid RP4 in Escherichia coli (E. coli) promoted the irreversible colonisation of polystyrene microplastics, which was speculatively suggested to be caused by the expression of conjugal pil. This suggests that HGT of ARGs, particularly via conjugation, may increase due to the nature of surface attachment, inducing the expression of HGT factors. In addition to this, Liu et al. (Liu et al., 2023a) found that exposure to smaller fractions of polystyrene microplastics (nanoplastics) enhanced the conjugal transfer frequency of RP4 in E. coli. When investigating the mechanisms behind this, it was revealed that the nanoplastic exposure induced reactive oxygen species and oxygen stress, which subsequently increased cell permeability of both donor and recipient cells, and upregulated the expression of mating pair formation genes and DNA transfer or replication genes. Microplastics themselves could therefore be described as co-selective agents for AMR, as the attachment to microplastics induces HGT machinery, or exposure to micro/nanoplastics results in the upregulation of conjugal transfer-related genes, thus indirectly selecting for an increased spread and genome incorporation of ARGs within Plastisphere communities. Furthermore, genetic linkage of AMR and virulence genes on multidrug resistant plasmids and virulence factors could also lead to the hitchhiking of AMR, due to the benefit conferred by the expression of virulence factors that support attachment.

Arias-Andres et al. (Arias-Andres et al., 2018) performed the first AMR experimental evolution study in the Plastisphere, conducting conjugation experiments on Plastisphere communities versus free-living cells and bacterial communities present on natural aggregates. Biofilms incubated in lake water were also evaluated for plasmid permissiveness using fluorescence-activated cell sorting. Plasmid transfer rates were three orders of magnitude higher on microplastics than those of bacteria in suspension, and the permissiveness of microplastic-associated communities was two orders of magnitude greater than bacteria in the surrounding water and natural aggregates (Arias-Andres et al., 2018). These results clearly demonstrate the elevated HGT potential of the Plastisphere compared with the aqueous phase, and begins to uncover distinctiveness to natural aggregates in the Plastisphere community's ability to receive and maintain AMR plasmids (Moradigaravand et al., 2022). Further to this, Feng et al. (Feng et al., 2023) found that conjugation rates of ARGs were significantly greater in the Plastisphere than compared with control wastewater communities, and that these ARGs could also be transferred horizontally to the free-living cells surrounding the microplastics. This postulates an additional concern where, not only may we see significantly greater HGT rates on microplastics, but this may also result in increased AMR in surrounding environments, including surface waters, if ARGs are indeed transferred from microplastics to the ambient, free-living community.

In addition to conjugation-based assays, the influence of microplastics on the transformation frequency of E. coli was also recently investigated by Wang et al. (Wang et al., 2022c), revealing that the presence of polystyrene nanoplastics increased transformation frequency significantly, but that microplastics had no significant impact. However, this study exposed E. coli to these particles and did not investigate transformation frequency within Plastisphere communities (see also Hu et al. (Hu et al., 2022b)). To the best of our knowledge, transformation within the Plastisphere had not been investigated until Wang et al. (Wang et al., 2023) recently investigated and compared...
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transformation frequency of ARGs in the Plastisphere, quartz sand biofilms and free-living counterparts. This work provides some of the first evidence to support a unique role of the Plastisphere in enhanced transformation-dependent HGT compared with natural substrate controls and free-living communities, finding that transformation frequency was significantly greater in the Plastisphere compared with both free-living and natural particle controls. The authors suggest that enhanced transformation in the Plastisphere was observed due to correlations between transformation frequency and bacterial density, extracellular polymeric substance content (including eDNA (Cheng et al., 2022; Zhang et al., 2022b)), expression of biofilm formation or DNA uptake genes (e.g. flagella motility and bacterial adhesion) and, at the community-wide level, greater colonisation of transformants. Furthermore, cell lysis of biofilm-housed cells has previously been observed as a mechanism to strengthen the structure of the biofilm, which suggests a further source of eDNA available for transformation (Abe et al., 2020).

To date, we are unaware of any investigation of transduction within Plastisphere communities, even though there is reason to suggest that elevated rates of this HGT mechanism would also be observed, due to the binding of bacteriophage proteins to plastics such as polystyrene (Bakhshinejad and Sadeghizadeh, 2016).

Given that many of these HGT mechanisms are experienced universally in biofilms, further research is required to identify the importance of microplastic substrates in comparison to natural materials. However, even with the current lack of studies adopting appropriate natural substrate controls, the uniqueness and potential increased risk of microplastics can still be proposed, given the ubiquity or longevity of microplastic particles in comparison with natural materials, co-occurrence with selective chemicals (Fig. 1) (Bydalek et al., 2023) or additional microplastic-dependent drivers (Fig. 2).
3.2. Adsorbed selective and co-selective compounds

Microplastics display elevated adsorptive capabilities compared to other natural debris owing to higher surface-to-volume ratios, and can achieve chemical loads over 100-fold greater than the surrounding water (Imran et al., 2019). Many pollutants found to accumulate on microplastics are responsible for selection or co-selection of AMR, including antibiotics, pesticides, biocides, heavy metals and other xenobiotics (Lagana et al., 2019; Rillig et al., 2018). Whilst the surface properties of microplastics (Fig. 2) or environmental conditions (e.g. salinity) partly facilitate the adsorption of these compounds, ageing (i.e. weathering, ultraviolet degradation of surface, biofilm formation) of microplastic particles has also been found to increase adsorption of pollutants (Bhatt et al., 2021). Though chemical contaminants can also adsorb to natural materials (Cederlund et al., 2016), it could be argued that microplastics more frequently come into contact with such pollutants given co-occurrences (Fig. 1), especially in environments polluted with sewage or landfill leachate (Shen et al., 2022).

Antibiotics are extremely susceptible to microplastic adsorption, which may result in the enhanced emergence, recruitment and fixation of ARGs in Plastisphere communities if these compounds are bioavailable (Du et al., 2022). To support this, a recent study found antibiotics (enoxacin, norfloxacin, ofloxacin and enrofloxacin) and various heavy metals adsorbed to macroplastic facemasks at significantly greater concentrations than stone controls (Liu et al., 2022d). Following further analysis, this work found that the selective pressure of the adsorbed co-contaminants led to the greater enrichment of ARGs in the Plastisphere compared to the biofilms attached to the natural, stone controls. Further examples of antibiotics shown to adsorb to microplastics include ciprofloxacin (Atugoda et al., 2020), sulfamethoxazole (Wang et al., 2022), oxytetracycline (Zhang et al., 2018), ampicillin (Zhao et al., 2021; Wang et al., 2021b), tetracycline (Ma et al., 2020), trimethoprim (Li et al., 2018), chloramphenicol (Wang et al., 2020) and roxithromycin (Zhang et al., 2022c). Yu et al. (Yu et al., 2022), for example, detected relatively high concentrations of antibiotics adsorbed to microplastics, including nearly 26 ng/g of the macrolide antibiotic spiramycin. Likewise, heavy metals have also been found to have a high affinity for microplastics, with concentrations reaching more than 600 times those present in surrounding waters (Yang et al., 2019b). Examples of heavy metals that have been previously documented to adsorb to microplastics include lead, cadmium, copper, zinc, chromium, iron, arsenic, silver and mercury (Khalid et al., 2021).

Very low, sub-inhibitory concentrations similar to those found adsorbed to microplastics have been previously shown to have AMR selective effects, including a study finding the minimal selective concentration for ciprofloxacin to be 100 pg/ml (Murray et al., 2018), suggesting that even low concentrations of antimicrobials present on microplastics may have a selective effect if they are bioavailable. In a study performed by Li et al. (Li et al., 2022c), polyvinyl chloride microplastics were pre-adsorbed with copper and/or tetracycline and incubated in an artificial activated sludge system. ARG diversity and abundance on microplastics and in sludge were then evaluated using metagenomics. The antimicrobials were found to distinctly enrich ARGs in the Plastisphere and surrounding sludge, particularly tetracycline resistance genes, supporting the role of microplastics in exerting a selective pressure due to adsorbed antimicrobials.

However, the role of selective concentrations of antimicrobial contaminants adsorbed to microplastics on the co-occurrence of antibiotic and metal resistance genes (Pal et al., 2015) driving co-selection for AMR on microplastics are yet to be fully understood, and the AMR selective risk posed by this is largely dependent on the bioavailability of these chemicals once adsorbed. Bioavailability of compounds adsorbed to microplastics has been previously contested. For example, the bioavailability of organic pollutants tocopepods was higher in the dissolved phase than when adsorbed to microplastics (Sørensen et al., 2020). However, this study did not account for the potential influence of microbacteria within the Plastisphere may have in increasing bioavailability of microplastic-associated chemicals through biodegradation, which is a potentially crucial yet understudied concern, highlighted below (Section 3.3).

3.3. Selective and co-selective compounds in the plastic matrix

Chemical additives are incorporated into the plastic matrix during the manufacturing process (Li et al., 2022c), with nearly 1000 different compounds currently associated with plastic polymers (Wright et al., 2020). Examples of some of these compounds include heavy metals like arsenic, cadmium, chromium and lead (Turner and Filella, 2021) or known antibacterial/antifungal agents, such as triclosan (Juncker and Hay, 2004). Some of the services provided by these additives include performance enhancement, increased durability, plasticisers, antioxidants, antifoulants, antimicrobials, ultraviolet and thermal stabilisers, colour pigments and flame retardants (Richard et al., 2019; Massos and Turner, 2017; Hansen et al., 2019).

Though many of these chemicals are not chemically bound and leach from plastics due to environmental ageing (Hahladakis et al., 2018), it is unknown what proportion of these compounds are bioavailable to the Plastisphere communities, and if so, whether they increase selection or co-selection for AMR. For the most part, the wider biological effects of these additives are also unknown. However, a recent study found that polyvinyl chloride leachate exposure significantly increased ARG and virulence gene relative abundance in a marine bacterial community, compared to the no-plastic leachate control (Vlaanderen et al., 2023). In addition, Lu and Chen (Lu and Chen, 2022) exposed a farmland soil community to high density polyethylene microplastics containing phthalates, and used metagenomics to explore the shifts in community composition and relative abundances of ARGs. Leaching phthalates from microplastics had a greater influence on ARG prevalence than microplastic exposure alone (e.g., particles physically causing changes to soil porosity), demonstrating that plastic additives can drive the evolution of AMR.

Notably however, these studies did not investigate the impact of additives on AMR in Plastisphere communities and focussed only on impacts to the surrounding microbiome and resistome. Further research should thus target understanding the impacts of plastic additives specifically within the Plastisphere and the importance of these unique, microplastic-dependent interactions in driving AMR. For example, the physical, chemical or biological degradation of microplastics may result in the increased bioavailability of additives to the Plastisphere. Indeed, the Plastisphere itself may increase additive leaching through the enzymatic hydrolysis of microplastic hydrocarbon bonds. Bacteria capable of producing plastic-degrading enzymes, also known as hydrocarbonoclastic bacteria, have been previously documented in the Plastisphere (Kelly et al., 2022; Du et al., 2022; Liu et al., 2022d). The subsequent release of additives as a result of biodegradation has not yet been investigated, but it has been noted that microplastics may be more likely to release these compounds than macroplastics, due to shorter diffusion path lengths (Li et al., 2022d; Paluselli et al., 2018; Luo et al., 2020; Bandow et al., 2017).

Furthermore, some compounds included as plastic additives are known co-selective agents, including heavy metals. Turner et al. (Turner et al., 2019) found that concentrations of bromine, cadmium, chromium and lead on ~10 % of beached microplastics were ‘non-compliant’ or ‘potentially non-compliant’ under the present regulations on hazardous plastic waste. Similar findings were observed in beached microplastics, where bioavailable concentrations of cadmium, lead and bromine exceeded those estimated as acceptable in the local seabird diet (Massos and Turner, 2017). It was proposed that the origin of bromine was likely due to the addition of brominated flame retardants. Crucially, a simulated avian digestive tract study revealed that heavy metal additives were bioavailable from microplastics during seabirds’ digestion (Turner, 2017). This is of particular concern as seabirds are renowned for their
ingestion of plastic debris, and release of additives could co-select for AMR in seabird gut microbiomes. In addition, seabirds are already recognised as important sentinels of AMR in the environment and are also used as microplastic biosentinels (Morrison et al., 2014; Van Franeker et al., 2011).

Crucially, if microplastic leachates are bioavailable and exert an AMR selective pressure, this is a mechanism unique to microplastics and should be seriously considered when addressing ecotoxicological risk and the AMR co-selective nature of microplastics in environmental compartments.

4. Pathogens and the plastisphere

In addition to AMR, it is important to understand the role of the Plastisphere in supporting the adhesion of pathogenic bacteria, given the threat of AMR pathogens to both human and animal health. This has recently been reviewed (Kaur et al., 2021; Junaid et al., 2022; Bowley et al., 2021; Bowley et al., 2022), where the capability for microplastics to harbour a variety of potential pathogens was reported.

Biofilms in general play a role in bacterial pathogenesis (Parsek and Singh, 2003). For example, the inducing of attachment phenotypes may increase infectivity of microbes, due to an increase in functional diversity or metabolic responses and pathways (Lyons et al., 2010; Sun et al., 2020). As previously discussed, QS is known to be an important driver of biofilm formation and cell-to-cell communication (Kaur et al., 2021), and it is proposed that the change from planktonic to biofilm lifestyle is governed by QS systems (Salta et al., 2013). QS is also responsible for the control of virulence factors (e.g. Lami (Lami, 2019)), which suggests a direct link between biofilm formation and the expression of virulence. Virulence genes can also be associated with adhesion factors, leading to the expression of virulence proteins as a result of surface attachment (Kaur et al., 2021; Radisic et al., 2020). Inversely, whilst existing as sessile cells, biofilms do not express invasion or motility machinery (Feltman et al., 2001; Mahenthiralingam et al., 1994), which may reduce colonisation potential and subsequent infection of the host.

Some of the clearest evidence for pathogen enrichment on marine microplastics involves the colonisation of Vibrio species (Zettler et al., 2013; Kirstein et al., 2016; Laverty et al., 2016; Rodrigues et al., 2019; Curren and Leong, 2019; Foulon et al., 2016; Silva et al., 2019; Sun et al., 2022b; Tan et al., 2022; Lai et al., 2022). Using next-generation sequencing, Frère et al. (Frère et al., 2018) detected members of the Vibrio genus on nearly 80 % of their microplastic samples, yet not all Vibrio spp. are pathogenic and Vibrio biofilms have also been identified on various natural debris, including wood and glass (Bowley et al., 2021). Other studies show the Plastisphere can not only harbour potential human pathogens, including faecal indicator organism E. coli (Metcalfe et al., 2022; Rodrigues et al., 2019), but also potent animal pathogens, such as the fish pathogen Aeromonas salmonicida (Lai et al., 2022; Virsek et al., 2017). Strikingly, within the terrestrial environment, potential pathogens were found to be 12.4 times higher in Plastisphere communities than in the ambient soil (Zhu et al., 2022). If Plastisphere communities do indeed select for pathogens as detailed in these studies, the need for continued exploration of microplastic-associated AMR is even greater, in order to reduce the risks posed by microplastic-associated, multi-drug resistant pathogens on public health and important food systems (e.g., aquaculture).

5. Ecological implications

Microplastic ingestion has been reported in over 200 species, with biofilm formation thought to increase palatability and thus the likelihood of consumer ingestion (Reisser et al., 2014; Hodgson et al., 2018). If colonised microplastics are ingested, associated ARB, ARGs or adsorbed antimicrobials could detach, transmit or desorb into the host microbiome. Additionally, following the egestion of microplastics, members of the gut microbiota may be incorporated into Plastisphere communities and integrated into microplastic-laden faecal pellets (Cole et al., 2016).

There is currently no information available on whether gut microbiota are incorporated into the excrated biofilm, how long they can survive after egestion, or the role of subsequent ingestion of contaminated faecal pellets. However, studies have started to elucidate the impact of microplastic exposure on gut microbiomes in vivo, where perturbed microbiomes can have critical negative impacts upon the host. Xu and Yu (Xu and Yu, 2021) exposed earthworms to polystyrene nanoparticles and microplastics, finding higher abundances of ARGS present in earthworm guts treated with microplastics in comparison to nanoparticles. Furthermore, a freshwater fish model (Carassius auratus) was used to assess the co-influence of the antibiotic roxithromycin and microplastics on ARG distribution on the fish gut microbiome and found a near 70 % increase in the ARG sul1 from the combined exposure to the antibiotic and microplastics, which was a marked increase compared with the single microplastic treatment and roxithromycin alone (Zhang et al., 2022c).

These results suggest that the co-occurrence of microplastics and antimicrobial residues in environmental compartments may risk biosecurity by increasing ARG prevalence in reared food exposed to both pollutants, such as fish (Zhang et al., 2022c). This could result in higher risk of treatment failure in the reared organisms, in addition to the potential increased risk of transmission to humans through occupational exposure or via the food chain.

Finally, when considering the ecological impacts of adsorbed or incorporated antimicrobial compounds and microplastics, there are some critical points that must be considered. For example, adsorption kinetics of antimicrobial compounds, the degradation rate of antimicrobials in water/sediment versus those associated with microplastics, and the impact of microplastic biofilms in influencing these two processes. Previous works (Tian et al., 2023; Wang et al., 2022g; Fan et al., 2021; Sun et al., 2023; Zhou et al., 2022b; Shi et al., 2022a; Tang et al., 2022) have begun to elucidate these interactions, for example, Sun et al. (Sun et al., 2023) found an increased adsorption of oxytetracycline to biodegradable microplastics when a biofilm was present, in comparison to virgin microplastics. In addition, Shi et al. (Shi et al., 2022c) report that the main adsorption mechanisms of microplastics rely on hydrophobicity interactions, hydrogen bonding and electrostatic interactions, and ageing increases heavy metal adsorption to both non-biodegradable and biodegradable microplastics. Further work is now required, especially when comparing microplastics to natural controls and adopting environmentally realistic conditions and concentrations, to fully understand these mechanisms and the unique risk posed by microplastics.

6. Environmental risk assessment implications

The universal framework for determining the environmental risk of pollutants (including both microplastics and antimicrobial chemicals), is to perform an ‘environmental risk assessment’. This typically includes an exposure assessment, effect assessment and risk characterisation (Everaert et al., 2018). Traditionally, effect assessments will quantify ‘safe’ concentrations of a pollutant using ecotoxicological tests, with endpoints including reduced survival, reproduction, or growth. The concentration at which no adverse effect on a test organism is observed is known as the no observed effect concentration. A no observed effect concentration can then be used to generate a predicted no effect concentration through application of an assessment factor, which takes into account extrapolating data from lab to field, and the breadth, depth and quality of effect data available (CHMP, 2006). Ultimately, environmental risk assessments produce a risk quotient or risk characterisation ratio by dividing the predicted or measured environmental concentration by the predicted no effect concentration. Generally, if the risk quotient or risk characterisation ratio exceeds 1, then the risk of that pollutant is considered high under European Union legislation (EU,
There are areas for improving environmental risk assessment of antimicrobials, discussed elsewhere, e.g., Murray et al. (Murray et al., 2021) and Alejandro et al. (Alejandro et al., 2023). However, the monitoring, legislation and mitigation of antimicrobials in the environment seems to be adopted more widely by both industry and government than those for microplastics. For example, concentrations of chemicals (including antimicrobials) in wastewater across the UK are currently being monitored by the UK Water Industry Research and Environment Agency’s Chemicals Investigation Programme (UKWIR, 2020), and predicted no effect concentrations for both ecotoxicity and AMR selection risk are used to inform the EU Commission Water Framework Directory’s Watch List of potentially hazardous substances (Gomez Cortes et al., 2022).

On the other hand, the first environmental risk assessment performed on microplastics was conducted in 2018 (Everaert et al., 2018), followed by Burns and Boxall (Burns and Boxall, 2018) and then for the first time with measured environmental concentrations in 2021 (Adam et al., 2021). All three of these global environmental risk assessments found that the concentrations of microplastics detected in the environment were orders of magnitude lower than those reported to have adverse effects on test organisms. However, these assessments did not include the risk posed by microplastics as vectors for chemicals in the environment or the effect of plastic additives. Endpoints that are typically adopted in microplastic ecotoxicological tests include reduced feeding, reproduction and growth, or tissue inflammation and mortality (Burns and Boxall, 2018), and test species are varied, including arthropods (e.g. Calanus sp. (Cole et al., 2015)) and molluscs (e.g. Mytilus sp.) (Adam et al., 2021).

Interestingly, one issue that has previously been highlighted for antimicrobial environmental risk assessments is the lack of testing against target microorganisms. Yet, ecotoxicological effects of microplastic exposure have recently been tested against the bacterium Vibrio angillarum, where the bacterial culture was exposed to increasing concentrations of polystyrene microplastics, and a no observed effect concentration was generated at the concentration of microplastics that significantly reduced culturability of the bacteria (Gambardella et al., 2018). However, this does not consider any impact of microplastics on microbes associated with the Plastisphere.

This review has discussed some potential interactions between microplastics and antimicrobials in the environment that may exacerbate the existing ecological threats of these pollutants. These interactions are not currently considered in either antimicrobial or microplastic environmental risk assessment frameworks and so we recommend the following:

a. To consider the role of microplastics as vectors for chemicals and the effects of plastic additives.

b. To consider the mixture effects of antimicrobials and other potentially co-selective pollutants (e.g. microplastics).

c. To improve the monitoring of antimicrobials by considering those incorporated into plastics or adsorbed to the plastic surface.

d. To consider that antimicrobials may persist on microplastics (Liu et al., 2018), increasing half-life durations and potentially altering where and when risks are present.

7. Concluding remarks

This review highlights three potential drivers of AMR selection within the Plastisphere, including increased HGT and the elevated AMR selection or co-selection pressures due to the presence of adsorbed or impregnated compounds. Though the ability of microplastics to enrich AMR communities relative to other substrates remains inconclusive, the persistence and transportation potential of these vastly abundant particles which co-exist with existing AMR selective pressures is evident. Therefore, it is imperative to develop our understanding of AMR within the Plastisphere so that combined threats of microplastics, ARB, ARGs and antimicrobial pollutants across One Health sectors can be fully assessed.

8. Outstanding research questions

- Biofilm-forming species are inherently resistant to antibiotic chemotherapy, due to reduced antibiotic penetration and slower growth rates. Also, large numbers of antibiotic-susceptible cheats may be enriched in the Plastisphere if antibiotic-inactivating enzymes, e.g., beta-lactamases, are secreted into the extracellular polymeric substance. What is the role of the Plastisphere in the reduction of antibiotic efficacy and in the survival of susceptible and resistant human or animal pathogens?
- Are existing laboratory-based findings reproducible in more realistic environments with less optimal growth conditions?
- Is there greater acquisition of ARGs or de novo mutations in the Plastisphere than free-living phases, or natural substrate controls?
- Do nanoplastics have a role in supporting attachment of AMR bacteria, or the increased transmission of ARGs?
- Antimicrobials exist in complex mixtures in the environment. Do these behave synergistically or antagonistically in terms of driving AMR in the Plastisphere?
- Does transduction play a role in HGT of ARGs within the Plastisphere, as well as conjugation and transformation?
- What is the role of microplastics in the increased transport of AMR bacteria between different environments, owing to increased protection and thus survival within the extracellular polymeric matrix?
- If colonised microplastics are ingested, could associated ARBs, ARGs or adsorbed antimicrobials detach/desorb and affect the host microbiome?
- Following the egestion of microplastics: are gut microbiota incorporated into the excreted Plastisphere, how long do these bacteria survive after egestion, and what is the role of subsequent ingestion of contaminated faecal pellets?
- Are fungi and viruses important members of the Plastisphere?

CRediT authorship contribution statement

EMS wrote the main manuscript text and prepared the figures, and all authors reviewed them. All authors conceptualized the project, reviewed the manuscript and led the funding acquisition. AKM was responsible for project administration. AB, MC, PKL and AKM supervised the project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Wang, H., et al., 2023. Microplastics as an important microniche that may accelerate the spread of antibiotic resistance genes via natural transformation. J. Hazard. Mater. 132085.


Zhou, Z., et al., 2022b. Adsorption behavior of Cu (II) and Cr (VI) on aged microplastics in antibiotics-heavy metals coexisting system. Chemosphere 291, 132794.