

Pathogenic Hitchhikers on Microplastics: Ecological Risks and Knowledge Gaps Based on Published Scientific Literature from 2000 to 2021

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Abstract

Microplastics (MPs) are ubiquitous environmental contaminants that can serve as transport vehicles for pathogens, potentially spreading them into human settlements and posing a public health threat. In recent years, concerns have been raised about the potential hazardous effects of these “pathogenic hitchhikers” on human health. This review paper presents foundational information on pathogenic hitchhikers associated with MPs, based on a review of the literature published in scientific journals from 2000 to 2021. We used search engines such as Google Scholar and Mendeley to retrieve publications during those inclusive years. Over the past two decades, we identified only 57 published articles on human pathogenic microorganisms associated with MPs, underscoring this area of research as relatively novel and emerging. For the reviewed articles, the common polymer types associated with pathogens were polyethylene and polypropylene, while *Vibrio* spp. were the most commonly reported human pathogens present on MP surfaces. Albeit small in scale, these studies provide incontrovertible proof that MPs can act as vectors for disease spread and pose a threat to the ecosystem. Significant gaps, nonetheless, remain, particularly concerning their harmful effects on humans and other macro-organisms that may inadvertently ingest MPs harboring these ‘hitchhikers’. Addressing these gaps in future research is thus warranted to comprehensively understand the ecological roles and health risks posed by these hazardous contaminants on MPs and for informing relevant policy decisions.

Keywords: *microplastics, pathogenic hitchhikers, polyethylene, polypropylene, research gaps, Vibrio spp.*

The term 'plastic' is applied to a wide range of synthetic organic compounds produced by polymerization, making them very flexible and robust (Hammer, 2012). These characteristics render plastics helpful in almost all aspects of daily life in modern society. The primary commodity plastics are made of polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET) (Andrady & Neal, 2009). Weinstein et al. (2016) reported that most plastics become brittle over time, fragmenting into progressively smaller particles through degradation. There are different classifications of plastic degradation according to the agent causing it, such as biodegradation, photodegradation, thermo-oxidative degradation,

thermal degradation, and hydrolysis (Andrady, 2011). These processes lead to the formation of microplastics (MPs). The production and use of plastics worldwide have increased dramatically over the past decades (Geyer et al., 2017). Microplastics pose significant concern given that their distinctive characteristics facilitate their swift and wide dispersal throughout the environment (Peng et al., 2020).

Microplastics range in size from very fine sand to coarse silt (>5 mm) (Gregory & Andrady, 2004) and occur in various shapes, including foams, fragments, shafts, fibers, and flakes (Su et al., 2019). While primary MPs are intentionally produced in micro-sizes depending on their use (e.g., microbeads in care products, glitter),

secondary MPs are formed from the fragmentation of larger plastics (Golwala et al., 2021). Landfills are considered sources of MPs primarily affected by the increased amount of plastic waste, lifetime of plastic products, and polymer degradation (Su et al., 2019). Sewage sludge also serves as a source of MPs for the environment, predominantly through the application of biosolids on agricultural lands (Rolsky et al., 2017). Sites such as landfills and waste products like sewage sludge are important and are found everywhere in the community. Microplastics are abundant in aquatic environments, including beaches and oceans, freshwater systems such as rivers and lakes, and wastewater treatment plants (Arcadio et al., 2023; Gabriel et al., 2023; Rezania et al., 2018). Microplastics are also widely distributed in soils and terrestrial environments (Gonzales et al., 2024; Sarker et al., 2020). Thus, MP pollution is widespread and constitutes a threat to the well-being of the ecosystem, the food web, and ultimately the health of human beings (Peng et al., 2020)

Organisms with a range of feeding strategies can ingest and accumulate microscopic particles (Browne et al., 2007). Marine organisms such as seabirds, turtles and other reptiles, marine mammals, fish, and invertebrates were reported to ingest MPs (Ryan, 2016). Microplastics can also function as vectors for the dispersal of dangerous microorganisms such as pathogens (Kirstein et al., 2016). Microplastics are thus often mistakenly identified as potential prey or food which endangers the life of organisms that ingest plastic debris (Ryan, 2016; Similatan et al., 2023). Also, the attachment of dangerous microorganisms or "pathogenic hitchhikers" on MPs (Keswani et al., 2016; Pang et al., 2021; Zettler et al., 2013) could be a means for spreading disease (Garcia-Gomez et al., 2021), as these can serve as vectors for the dispersal of attached invasive species of microorganisms on plastic surfaces (Bowley et al., 2022). Pathogens associated with MPs can be classified as commensal (normally harmless residents), obligate (requiring a host to cause disease), or opportunistic (causing illness under favorable conditions)—depending on their ecological roles and pathogenic potential (Madigan et al., 2021). However, the mechanisms for pathogen transmission from plastic to macroorganisms such as humans are still not well understood (Zhong et al., 2023) and require deeper research using cutting-edge technologies.

The global distribution of MP fragments has continually increased over the past few decades (Barnes et al., 2009). As plastic debris in the environment continues to increase, the potential for MPs to act as vectors for pathogen transport has

become an emerging concern (Bowley et al., 2022). The dispersal of invasive species could endanger sensitive or at-risk coastal environments, both marine and terrestrial (Gregory, 2009).

To combat environmental problems such as plastic pollution, science-based decision-making is fundamental (Onda et al., 2020). Research findings must guide government policies to ensure environmental sustainability and public health safety. Thus, research investigations on the association of dangerous microbial hitchhikers with MPs warrant attention from the scientific and non-scientific communities. Plastics and microbial interactions have been documented as early as the 1970s (Onda & Sharief, 2020), but the main drivers of these interactions, the distribution of pathogens on MPs, the impact of the pathogens on human health through MPs, transmission routes and infection probability remain unclear to this day (Zhong et al., 2023).

A comprehensive review of the literature on pathogenic hitchhikers is necessary to understand and build foundational information on pathogenic hitchhikers, such as their dispersal, transmission through MP and infection risks, and guide future studies aimed at safeguarding human welfare and environmental health in the face of rising MP pollution. Thus, the primary objective of this study was to determine foundational information about pathogenic microorganisms associated with MPs using a systematic review of scientific literature, with specific attention given to the following information: (i) the proportion of articles concerned with human pathogenic hitchhikers on MPs from the year 2000 to 2021, (ii) the identity of potential human pathogenic microbial hitchhikers that are associated with MPs, and (iii) the polymer type of MPs where potential human pathogenic hitchhikers are associated. The second objective was to present the possible impact of pathogenic hitchhikers on the ecosystem, providing evidence gleaned from the research articles reviewed. In this study, we are also able to highlight gaps in knowledge in the area of microorganism-MP science which can point to topics that need to be prioritized in research towards understanding the ecological role and the health risks associated with these hitchhikers. The literature analysis was conceptualized and completed during the height of the COVID-19 pandemic, when access to resources, research continuity, and project timelines were significantly constrained. As the study was conducted within a fixed academic schedule, the review intentionally focused on literature published from 2000 to 2021. Limiting the scope to this period ensured that the analysis was both manageable and methodologically coherent, allowing for a thorough

and systematic synthesis of two decades' worth of foundational research. Focusing on this timeframe also allowed the study to capture the core developments, trends, and baseline knowledge that shaped the early trajectory of MP research.

Materials and Methods

Article Selection

From December 2021 to January 2022, publications were retrieved through searches in Google Scholar and Mendeley. A trial search for this topic for the period 1990–1999 revealed no relevant articles; thus, only articles from 2000 could be included. Also, it was in 2000 that the dangers and risks of plastic pollution started to gain attention (Onda & Sharief, 2020). The following keywords were used: microplastics, dangerous hitchhikers, biofilms, plastisphere, and human pathogens. All articles selected belonged to a peer-reviewed journal. To confirm this, each journal was reviewed individually by visiting its official website, which contains information on its peer review process.

The criteria for selecting articles to assess the proportion of studies addressing human pathogenic microorganisms associated with MPs required that the articles explicitly discuss human pathogenic hitchhikers on MPs as part of their scope. Each article underwent a two-stage screening process to determine its relevance. In the

first stage, articles were evaluated based on keywords and abstracts. Articles that met the initial criteria were then downloaded for further review. In the second stage, the full text of each article was examined in detail after thorough reading, with a focus on its objectives, methodology, and results and discussion. Additionally, the reference lists of the selected articles were scrutinized, and the relevant articles identified therein were subjected to the same screening process. To determine the identity of potential human pathogenic hitchhikers associated with MPs and polymer type, article selection was based on the following criteria: (i) samples were extracted from biofilms associated with MPs, and (ii) DNA sequencing technology was used to confirm the identity of the microorganisms.

Data Collection and Analysis

The gathered data from the systematic scrutiny of the articles were interpreted and analyzed. Articles that passed the previously mentioned criteria were categorized based on the type of research: (i) Review (RE), survey/discussion of scholarly works, (ii) Characterization of Microbial Community (CMC), basic profiling or characterization of microbial community associated with MPs, or (iii) Experimental Works (EW), studies that included a variable that can be manipulated by the researcher and tested in a controlled environment. General information about

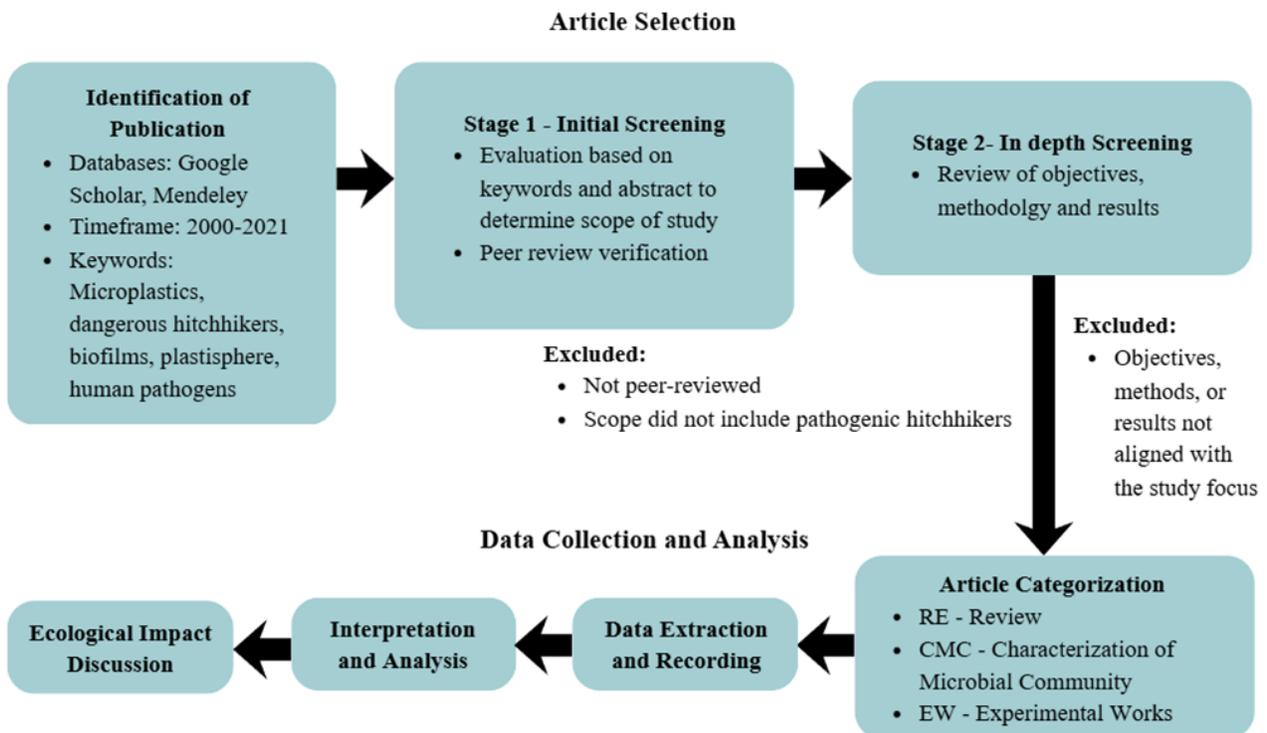


Figure 1. Overview of the methodology on article selection and data collection and analysis

the paper such as author and year of publication, general methodology, and main results were recorded and organized in a raw Microsoft Excel table. The possible impact of pathogenic hitchhikers on the ecosystem was discussed based on the presented information in the relevant articles. This was categorized by themes such as plants, animals, and humans.

Results and Discussion

Research on Human Pathogenic Hitchhikers on MPs in 2000-2021

The number of published articles that were focused on microbial hitchhikers, particularly human pathogens on MPs, was scanty, but has increased significantly in later years (Figure 2). In this review, only 57 articles on this topic were retrieved from 2000 to 2021. This low number of articles can be ascribed to the difficulties entailed in studying MPs. Similar views were espoused by Peng et al. (2020), who stated that MPs had been largely ignored in most studies due to their elusive nature as well as limitations associated with sampling and analytical protocols. Furthermore, these difficulties were exacerbated by the introduction of the "plastisphere" concept (Onda & Sharief, 2020).

In our review, the first retrieved publication about microbial hitchhikers on MPs was published in 2013 (Figure 2). This article was authored by Zettler et al. (2013), where the 'plastisphere' was first described. It highlighted the potential of marine plastic debris to serve as a new environment

for microorganisms. Several studies followed, which reported similar conclusions. One study by McCormick et al. (2014) investigated the bacterial assemblages present in MPs collected from an urban river. A year later, a comparative study of bacterial assemblages on MPs collected from beaches, sediments, and seawater came out (De Tender et al., 2015). The above studies revealed that plastics could act as vehicles for a wide variety of microorganisms.

It was also observed that the number of publications started to increase significantly starting in 2019 (Figure 2). This is supported by the article review by De Tender et al. (2017), which provides an overview of different techniques for plastisphere studies. These techniques included molecular and visualization methods for characterizing life in the plastisphere. With the increasing standardization of techniques, it can be expected that plastisphere studies will continue to increase in the following years. This is revealed by a relatively higher number of publications in the succeeding years 2020 and 2021.

The geographical distribution of the recovered articles shows that most studies were conducted in Asia and Europe. Seventeen studies (Jiang et al., 2018; Gong et al., 2019; Huang et al., 2019; Wu et al., 2019; Li et al., 2019; Shen et al., 2019; Wang et al., 2020; Sun et al., 2020; Feng et al., 2020; Zhou et al., 2020; Yang et al., 2021; Meng et al., 2021; Hou et al., 2021; Zhao et al., 2021; Zhu et al., 2021; Dong et al., 2021; Shen et al., 2021) originated from China, which is currently the world's largest plastic producer, accounting for

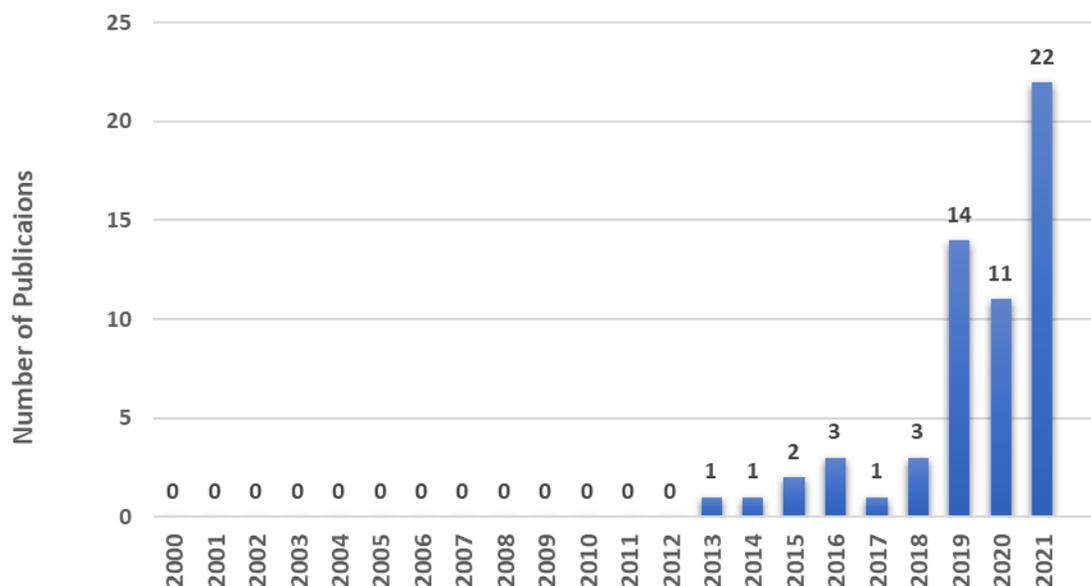


Figure 2. Number of publications per year (for the period 2000-2021) concerned with human pathogenic hitchhikers on MPs in publicly available databases of scientific literature.

26% of global plastic production (Peng et al., 2017), and a major contributor of plastic inputs to the ocean through rivers, estimated at 70,707 metric tons per year (Meijer et al., 2021). Eight studies (Oberbeckmann et al., 2015; Kirstein et al., 2016; Oberbeckmann et al., 2018; Oberbeckmann and Labrenz 2019; Kesy et al., 2019; Song et al., 2020; Moresco et al., 2021; Oberbeckmann et al., 2021) were from Germany. Germany is one of the world's major plastic exporters and producers of total plastic waste (Ritchie & Roser, 2018). The prominence of studies from these countries aligns with their established attention to plastic production, waste management, and pollution, which has been reflected in policies such as early plastic awareness campaigns in parts of Europe during the 1970s (e.g., the "Jute not Plastic" initiative) (Bruns & Sommer, 2020) and China's "plastic limit order" in 2008 followed by more

comprehensive regulations in 2020 (Liu et al., 2021). These historical and regulatory contexts may have contributed to the increased scientific focus on plastic pollution in these regions.

In all articles recovered in this review, different research types were identified. Of the 57 articles, experimental works accounted for the majority (41%), followed by review articles (33%) and articles characterizing microbial communities on MPs (26%) (Figure 3A). Those categorized as Reviews (RE) were surveys or discussions of scholarly works. Studies under the Experimental Works (EW) category included variables manipulated by the researcher and conducted testing in a controlled environment. On the other hand, under the Characterization of Microbial Community (CMC) category were studies that have undergone basic profiling or characterization of the microbial community associated with MPs.

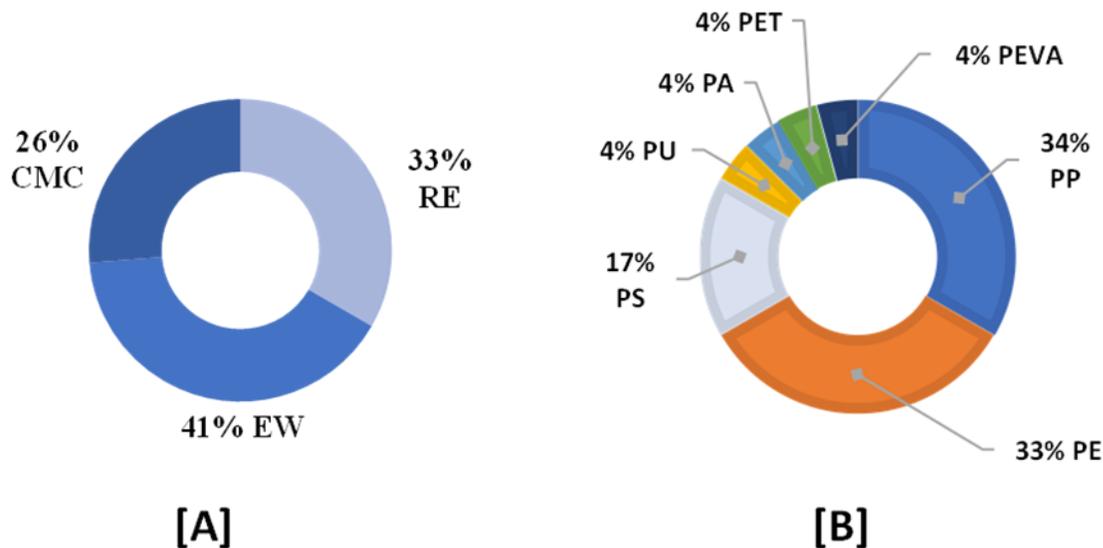


Figure 3. [A] Percentage of the 57 recovered articles under three categories of research type. Articles were categorized as Experimental work (EW), Review (RE), and Characterization of Microbial Community (CMC). [B] Polymer types of MPs with associated potential human pathogens reported by recovered scientific articles published in 2000-2021. PP – polypropylene; PS – polystyrene; PU – polyurethane; PE – polyethylene; PA – polyamide; PET – polyethylene terephthalate; PEVA – polyethylene vinyl acetate.

Twenty-three (23) or 41% of the articles retrieved were experimental works (Figure 3A). Eight of these aimed to investigate the differences in microbial community structure between biofilms on MPs and on natural substrates. To do so, Wu et al. (2019) incubated biofilm on polyvinyl chloride (PVC) MP pellets and two natural substrates, such as rocks and leaves, under a controlled environment. Through incubation experiments, other comparisons were done between polyethylene (PE) and polystyrene (PS) MP biofilms and

biofilms of wood and seston (Kesy et al., 2019), biofilms of MPs and feathers (Sun et al., 2020), and biofilms of high-density polyethylene (HDPE) on wood and on tire wear (Song et al., 2020).

All studies have similar findings: MPs biofilms have unique microbial community structures. The authors also concluded that compared to natural substrates, MPs can be carriers for the enrichment of dangerous microorganisms in the environment, which might increase the ecological risk of MPs. In Kesy et al. (2019), for

instance, bacterial assemblages analyzed via 16S rRNA-gene amplicon sequencing, distance-based redundancy analyses, and linear discriminant analysis effect size method revealed that Alphaproteobacteria (*Sphingomonadaceae*, *Devosiaceae*, and *Rhodobacteraceae*) and Gammaproteobacteria (*Alteromonadaceae* and *Pseudomonas*) were distinctive for the PE- and PS-associated biofilms. *Vibrio* was more abundant in the PE and PS biofilms, with the highest abundance in wood and a positive correlation with salinity. These experimental works are essential in providing empirical evidence that MPs should be of concern due to their ability to serve as vectors for the dispersal of dangerous microorganisms, which was reiterated in later works by Bowley et al. (2022), Cholewińska et al. (2022), Zadjelovic et al. (2023), and Zhong et al. (2023), among many others.

The nineteen (19) review articles, which constituted 33% of the articles obtained (Figure 3A), delved into the existing knowledge and research gaps related to MPs and microbial hitchhikers' association with MP. Caruso (2019), Imran et al. (2019), Stenger et al. (2021), García-Gómez et al. (2021), and Marathe and Bank (2022) discussed MPs as a medium for the dispersal of chemical and biological contaminants such as antibiotic resistance genes and antibiotic-resistant pathogens, including *Vibrio* sp. (Laverty et al., 2020; Zhang et al., 2020) and *E. coli* (Song et al., 2020).

Shen et al. (2019) and Meng et al. (2021) found that MPs can support horizontal gene transfer, thereby increasing gene exchange between attached biofilm communities. Bowley et al. (2020) further discussed the threats of the interaction between MPs and dangerous microorganisms, revealing growing evidence that MPs represent a potential reservoir of pathogens and antibiotic-resistant bacteria that differ markedly from natural particles. In contrast, Oberbeckmann and Labrenz (2020) emphasized that MPs pose no greater risk than natural particles. This was based on their analysis that the majority of microorganisms growing on MPs seems to belong to opportunistic colonists who do not distinguish between natural and artificial surfaces. Several research gaps and recommendations were presented and addressed in these review articles. First, Moresco et al. (2021) highlighted the importance of tackling sources of MP pollution to decrease the potential for pathogens to become bound and transported through the environment. Second, a comprehensive assessment of the potential for MPs to serve as transmission vectors of harmful microorganisms is needed (Keswani et al., 2016; Oberbeckmann et al., 2015). Third, it has been

emphasized in review articles that future research must entail studying microbial and plastic interactions at all accumulation sites. Unlike previous review articles, this study integrates two decades of literature to provide a consolidated, cross-ecosystem synthesis of pathogenic hitchhikers on microplastics, highlights understudied ecological pathways across humans, plants, and animals, and identifies research gaps that have not been comprehensively mapped in earlier reviews.

Fifteen (15) articles, or 26% of the articles retrieved, characterized the microbial community on MPs (Figure 3A). Microplastic samples were collected from different locations such as ocean waters (Lacerda et al., 2019; Zettler et al., 2013), sea waters (De Tender et al., 2015; Kirstein et al., 2016), rivers (McCormick et al., 2014; Yang et al., 2021), coastlines (Curren & Leong, 2019; Radisic et al., 2020; Dong et al., 2021), lakes (Tavsanoglu et al., 2020), beaches (Rodrigues et al., 2019), and estuaries (Jiang et al., 2018). Using DNA sequencing, these studies identified human pathogenic strains of *Vibrio* spp. and *E. coli*. Identification and characterization of the composition of microorganisms present in MPs are thus crucial in providing a foundation for understanding their ecological function (Onda & Sharief, 2020). This can also provide insights into the risks of MPs to the environment and their ecology.

MP Polymer Types Associated with Potential Human Pathogens

The common polymer types that potential human pathogens are associated with based on their mention or use in the retrieved articles are polypropylene (PP) (34%), polyethylene (PE) (33%), and polystyrene (PS) (17%) based on 12 studies that qualified the selection criteria with minor proportions for polyethylene terephthalate (PET), polyurethane (PU), and polyethylene vinyl acetate (PEVA) (Figure 3B; Table 1). Amaral-Zettler et al. (2015) and Keszy et al. (2019) suggested that polymer type plays a less significant role in biofilm formation, whereas environmental conditions are thought to be the main drivers of the composition of the microbial community on MPs. However, Frere et al. (2018) found that the polymer family significantly influences the composition of the MP bacterial community. PS-type presented a bacterial community distinct from that of PE and PP. It appears that bacteria show selectivity for the type of plastic debris and tend to colonize their preferred substrate (Li et al., 2019). Microorganisms tend to attach to surfaces based on the advantages those surfaces offer (Zhong et al., 2023) and MP properties most

important to this are hydrophobicity, texture, electrical charge, and biodegradability. Zhong et al. (2023) further cited studies indicating that specific microbes, including pathogenic strains, are often found in greater numbers in MPs than in non-plastic substrates. For instance, families *Hyphomonadaceae* and *Erythrobacteraceae* were found to be significantly more abundant on PS and PE than on wood pellets (Oberbeckmann et al., 2018), and *Burkholderiales* were more than twice as high on MPs as on other materials (Ogonowski et al., 2018). Additionally, MPs can have a marked accumulation of antibiotic resistance genes (ARGs), as they generally exhibit higher average absolute abundances of these genes than nearby sediment samples (Guo et al., 2020). Thus, investigations focused on the effect of polymer type on the composition of microbial communities on its surface are needed to deepen understanding of the impact of MP-associated microbial assemblages on their environment. If polymer types have a role in the composition of microbial communities on MPs, the presence of human pathogens on MPs could increase the threat to public health. This is because PE and PP, the common polymer types with associated potential human pathogens, are abundant in the environment. According to studies investigating plastic pollution, PP and PE are widespread especially in marine environments such as the sea surface and beach sediments, rivers, and the ocean (Digka et al., 2017; Xu et al., 2021; Zhang et al., 2020). Thus, the presence of PP and PE in the environment indicates the potential presence of human pathogens. The transmission mechanism from MP to humans and animals, though, is poorly understood at this time.

Potential Human Pathogens Associated with Microplastics

Twelve studies met all the criteria for articles reporting the identity of potential human pathogens on MPs and the polymer type to which these pathogens are associated (Table 1). The common genus found in these studies was *Vibrio*. However, data collected from most studies were not enough to detect and identify *Vibrio* at the species level. Only two (Curren & Leong, 2019; Silva et al., 2019) of the 12 retrieved studies detected *Vibrio* at the species level. In the study by Curren and Leong (2019), high-throughput sequencing revealed a rich bacterial assemblage on particle surfaces. One of the pathogens identified as a community component was *Vibrio fluvialis*. This species is a known emerging pathogen commonly found in coastal environments (Ramamurthy et al., 2014). It is an essential cause of cholera-like bloody diarrhea and wound infection (Igbinosa &

Okoh, 2010). Furthermore, Curren and Long (2019) found that the microbial community structures differed largely at the phylum level among their sampling sites. *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were the dominant phyla, which are consistent with other microbial community characterization on the surfaces of marine MPs (Amaral-Zettler et al., 2015; Oberbeckmann et al., 2015).

In another study by Silva et al. (2019), *E. coli* and *Vibrio* spp. were detected from water and floating plastic debris samples collected from various sites in Guanabara Bay in southeastern Brazil, in the Rio de Janeiro state, using biochemical tests and PCR to identify *E. coli* virulence serovars and to detect the virulence genes. *Escherichia coli* serotypes bearing the virulence genes EaeA (for EHEC or EPEC), iAL (for EIEC), stx (for EHEC), and lt (ETEC) were revealed in their analysis as well as three *Vibrio* spp.. One of the identified species was *V. cholerae*. This species colonizes the small intestine and produces an enterotoxin, cholera toxin, which causes cholera if ingested (Faruque et al., 1998). *Vibrio cholerae* can survive, colonize, and express virulence factors despite and in response to harsh environmental conditions (Reidl & Klose, 2002). Another *Vibrio* sp. detected was *V. vulnificus* which is an opportunistic human pathogen that causes severe, fulminant systemic infection (Jones & Oliver, 2009). *Vibrio mimicus* was also detected in the study by Silva et al. (2019). Acute gastroenteritis following ingestion of seafood (especially raw oysters) and acute otitis after exposure to seawater are diseases attributed to *V. mimicus* (Shandera, 1983). The summarized information in Table 1 shows that *Vibrio* was detected from MPs collected from salt environments like oceanic waters, seawaters, coastlines, and beaches. *Vibrio* species are halophilic or salt-loving (Morris, 1988) and are found in all ocean environments, ranging from coastal to open and surface to deep water (Thompson et al., 2004).

Pseudomonas has also been detected on MPs collected from salt and freshwater environments (Table 1). This is because *Pseudomonas* can adapt to a wide range of habitats and live in them (Mena & Gerba, 2009). This genus also includes human pathogenic species such as *P. aeruginosa* which is most commonly associated with ear and skin infections (Mena & Gerba, 2009).

Certain *Vibrio* sp. and *Pseudomonas* sp. cause life-threatening illnesses; thus, their presence on the surfaces of MPs can be a threat to humans. Exposure to these MPs may endanger public health, as MPs are present in bays and beaches open to human activities. Sun and Wang (2023)

Table 1. Summary of the published articles 2000-2021 reporting the presence of potential human pathogens on microplastics.

Reference	Potential Human Pathogen	Polymer Type*	Detection Method	Location
Jiang et al., 2018	<i>Vibrio</i> <i>Pseudomonas</i>	PP, PE, PS	High throughput sequencing (16s rRNA gene)	Intertidal Zone
Curren & Leong, 2019	<i>Arcobacter</i> <i>Vibrio fluvialis</i> <i>Pseudomonas</i>	undetermined	High throughput sequencing (16s rRNA gene)	Beaches
Dong et al., 2021	<i>Vibrio</i> <i>Pseudomonas</i>	PP, PE	High throughput sequencing (16s rRNA gene)	Marine Aquaculture sites
Yang et al., 2021	<i>Pseudomonas</i> , <i>Acinetobacter</i> , <i>Serratia</i> , <i>Clostridium</i>	undetermined	Illumina sequencing of the 16s rRNA gene libraries	Riverine waters
Frere et al., 2018	<i>Vibrio</i> spp.	PP, PE, PS	High throughput sequencing (16s rRNA gene); RT-PCR analysis	Bay
McCormick et al., 2016	<i>Arcobacter</i>	PE, PP, PS	High throughput sequencing (16s rRNA gene)	Urban River
Lacerda et al., 2021	<i>Alexandrium tamarense</i> <i>Vibrio</i>	PA, PU, PE, PP, PS	High throughput sequencing (16s rRNA gene)	Coastal and oceanic waters
Silva et al., 2019	<i>Escherichia coli</i> <i>Vibrio</i> spp. (<i>V. cholerae</i> , <i>V. vulnificus</i> , <i>V. mimicus</i>)	PE, PP, PET	Polymerase chain reactions	Bay
Radisic et al., 2020	<i>Morganella morganii</i>	PE, PEVA	Whole genome sequencing	Coast
McCormick et al., 2014	<i>Aeromonas</i> , <i>Arcobacter</i> , <i>Pseudomonas</i>	undetermined	High throughput sequencing	Urban River
Zettler et al., 2013	<i>Vibrio</i>	PP	Next-generation sequencing	Ocean
De Tender et al., 2015	Vibrionaceae	PP, PE	High throughput sequencing	Sea

* PP- polypropylene; PS- polystyrene; PE – polyethylene; PET – polyethylene terephthalate; PU- polyurethane; PEVA - polyethylene vinyl acetat

illustrated three exposure routes of MPs in humans – via ingestion, inhalation, and dermal penetration, with ingestion considered as the primary route. Because of the magnitude of MP contamination in the oceans (estimated average of approximately 6300 MPs per km² or 0.03 MPs per m³ float on the ocean surface), seafood may be the primary source of MPs by the ingestion route (Bohdan, 2022).

Potential Impact of Pathogenic Hitchhikers on the Ecosystem

Plants. Plant pathogens were found on MPs. Using ITS metabarcoding, comprehensive meta-analysis, and visualization techniques, combined with scanning electron and confocal laser scanning microscopy, pathogenic and non-pathogenic fungi, including the rare plant pathogens *Leptosphaerulina australis*, *Hannaella oryzae*, and *Phaeosphaeria podocarpi*, were detected on MPs collected from municipal plastic

waste in Kenya (Gkoutselis et al., 2021). The plastisphere core mycobiome (PCM) was profiled in this study, revealing that approximately 20% of the molecularly identified phylotypes were unique to plastic rather than to soil mycobiomes. Similarly, in the study by Wu et al. (2019), the plant pathogen, *Pseudomonas syringae*, was detected only in the MP biofilm, but not in biofilms formed on natural substrates of rock and leaf. This plant pathogen can infect almost all economically important crop species (Xin et al., 2018). Thus, MPs' ability to transport plant pathogens into crop fields may degrade crop quality, reduce yields, and ultimately result in significant economic losses (Wu et al., 2019). In addition, human pathogenic microorganisms that can be carried on or in the plant tissue, either as transients or true epiphytes, may cause disease when introduced to immunocompromised individuals (Mendes et al., 2013).

Animals. Putative animal pathogens were found on plastic debris in several investigations. Using next-generation sequencing, the fish pathogen *Tenacibaculum sp.* and invertebrate pathogens *Phormidium sp.* and *Leptolyngbya sp.* were detected on collected plastic debris in the Western Mediterranean Sea (Dussud et al., 2018).

Another bacterial fish pathogen *Aeromonas salmonicida* was also detected on MPs collected in the North Adriatic Sea via PCR amplification of 16S rDNA (Virsek et al., 2017). In the eastern Pacific, the coral pathogen *Halofolliculina spp.* was identified from plastic debris collected in the eastern Pacific and western Pacific oceans (Goldstein et al., 2014). Thus, a wide variety of animals are at risk due to the possibility of ingesting MP coated with pathogens. MPs associated with dangerous microorganisms could thus transfer to higher trophic levels, posing a risk to the overall ecosystem. However, the ecological role of plastic-associated assemblages in the open ocean remains unclear (Gkoutselis et al., 2021). One thing was evident in the study by Goldstein et al. (2014): the potential impact of debris-associated microorganisms in pelagic ecosystems can be mitigated by reducing plastic pollution in the marine environment.

Humans. The main route of transmission of human pathogens such as *Vibrio* to humans is through the consumption of inadequately cooked seafood or aquatic food products (Osunla & Okoh, 2017). Seafood consumption represents one pathway for human MP exposure (Smith et al., 2018). It was found that seafood is among the top three contributors to human consumption of MPs (Cox et al., 2019). Microplastics were also detected from human stool samples collected from participants who consumed seafood during the observation period (Schwabl et al., 2019).

The aquaculture industry has been a major source of seafood for human consumption (Little et al., 2016). Marine diseases of farmed oysters, shrimp, abalone, and various fish are one of the biggest issues the industry encounters (Latva et al., 2021). Several *Vibrio* species are considered important pathogens and have provoked severe mortality outbreaks, affecting shellfish production (Beaz-Hidalgo et al., 2010). The presence of these pathogens on MPs adds to this concern. Aquatic animals such as bivalves are at risk of MP ingestion due to their filter-feeding nature, which exposes them directly to MPs in the environment. In fact, there is evidence showing that bivalves such as mussels can ingest MPs (Browne et al., 2008; Van Cauwenberghe & Janssen, 2015).

In the investigation conducted by Fabra et al. (2021), the uptake and bioaccumulation of virgin or uncoated MPs and *E. coli*-coated MPs by

European native oysters (*Ostrea edulis*) were compared. It was found that the uptake of *E. coli*-coated MPs was significantly higher than the uptake of virgin MPs in oysters. In addition, the oxygen consumption and respiration rate of oysters exposed to *E. coli*-coated MPs increased dramatically over time, whilst virgin MPs did not. It provides compelling evidence to support that MPs can function as pathogen vectors through organisms, or at least through bivalves.

It has been emphasized that seafood consumption is a route for both the transmission of pathogens and MPs. Growing scientific evidence highlights several pathways for MP exposure through food, including findings that MPs are present in species important to global marine fisheries (Lusher et al., 2017). According to Smith et al. (2018), emerging evidence from studies on toxicity and epidemiology suggests that MPs, due to their association with harmful chemicals, pose potential health risks to humans, particularly through the consumption of seafood contaminated by these particles. Thus, there is a risk of human exposure to pathogens associated with MPs, with seafood consumption as a probable route. Attention to the mechanisms must now be given through research, as it is still unclear how MPs affect human biological tissue. Therefore, investigations about the potential human intestinal absorption of MPs and their effects on human health are also warranted.

Gaps in Research on Microplastics and Their Microbial Hitchhikers

Beneficial microbes in MPs. Studies on the impact of plastic debris have increased in the last decade, but studies on the plastisphere are just (Onda & Sharief, 2020; Roager & Sonnenschein, 2019). Most plastisphere studies focus specifically on characterizing the community structure of the colonizing organism (Latva, 2021). The hydrophobic nature of plastic surfaces promotes the formation of a microbial biofilm, an assemblage of surface-associated microbial cells enclosed in an extracellular polymeric substance matrix (Donlan, 2002). The associated microbial community may be composed of heterotrophs, autotrophs, predators, and symbionts, and is collectively referred to as the plastisphere (Zettler et al., 2013) where many species found may be harmful and hence, called “dangerous hitchhikers” (Kirstein et al., 2016; Zettler et al., 2013). The plastisphere could thus be a reservoir for pathogenic microbes, fecal contaminants, and harmful algal bloom species. Microplastics can also carry antibiotic-resistant bacteria (Pham, 2021). Plastics may serve as alternative sources of food and energy (Romera-Castillo et al., 2018) that can support the growth

and survival of microorganisms during transport. The microorganisms associated with plastics are thus functionally and physiologically diverse. This also suggests that the community composing the plastisphere may include beneficial components, such as those with plastic-degrading properties. This is illustrated in the study of Delucavellerie et al. (2019). From floating plastics and sedimentary plastics in the Mediterranean Sea, hydrocarbon-degrading bacteria such as *Alcanivorax*, *Marinobacter* and *Arenibacter* were enriched with low-density polyethylene (LDPE) and polyethylene terephthalate (PET), implying that these bacteria have potential roles in plastic degradation. Nevertheless, research on beneficial microbes associated with MPs in aquatic and terrestrial ecosystems is currently limited, highlighting a significant gap. There is a need for more comprehensive studies on the ecological roles of these microbes, particularly their interactions with MPs that affect nutrient cycling and ecosystem health. Specifically, understanding the mechanisms through which microbes degrade MPs and their potential role in bioremediation strategies requires further exploration. In terrestrial ecosystems, the impact of MPs on soil microbial communities and their functional diversity remains poorly understood, limiting insights into soil health and agricultural productivity. Research on terrestrial ecosystems has primarily focused on single-species interactions, neglecting the dynamics of microbial communities that could elucidate their roles in nutrient cycling and ecosystem services. Additionally, the potential for MPs to act as selective environments for beneficial microbes, particularly fungi, is underexplored. Future research should prioritize multi-species studies in realistic environmental settings to comprehensively assess the ecological impacts of these interactions. Understanding these dynamics is crucial for developing strategies to mitigate the environmental risks posed by MPs. There is also a substantial need for studies investigating the effects of MP-associated biofilms on microbial dynamics and the potential positive roles that beneficial microbes may play in mitigating MP pollution. Addressing these research gaps can significantly advance our understanding of microbial interactions with MPs and contribute to effective ecological management strategies.

Persistence of pathogenic microbes on MP substrates. Beaches are one of the most recognizable marine ecosystems. These serve as important habitats for many animals, and this is also where people spend time for different leisure activities. Studies providing evidence for the presence of dangerous and potentially pathogenic microorganisms associated with MPs on beaches

were presented in this literature review. These point out beaches as areas where plastics can serve as vectors for dispersing pathogenic microorganisms. According to Keswani et al. (2016), the ability of microorganisms to persist on plastic debris found on beaches needs urgent addressing so that regulators and beach managers could make more informed decisions about public safety in bathing environments.

Drivers of horizontal gene transfer in terrestrial MP-microbe communities. Several studies have reported the composition and diversity of plastisphere microbiota associated with MPs in aquatic ecosystems. Still, there are limited studies about the plastisphere microbiota associated with MPs in terrestrial environments. Zhu et al. (2021), for instance, investigated plastisphere microbiota and antibiotic resistance genes (ARGs) of four types of MPs from diverse soil environments through three microcosm experiments and one field experiment. Using the Smart Chip Real-Time PCR System to analyze extracted DNA, a range of potential pathogens and ARGs were detected in the plastisphere. Potential human pathogens such as *Burkholderia sp.* (5.2%) and other pathogens such as *Bordetella avium* (34%) and *Pseudomonas fluorescens* (13%), *P. protegens* (6.8%), and *P. syringae* (5.1%) were detected. In another study, the aging and hetero-aggregation of a microplastic sampled in farmland soil was characterized, and likewise, ARGs for tetracycline, beta-lactam, and sulfonamide resistance were detected on the plastic surface in the soil ecosystem (Yan et al., 2020). Thus, the plastisphere associated with MPs found in soils may represent a hotspot of increased potential for horizontal gene transfer. This may accelerate the spread of antimicrobial resistance globally and impose danger also in non-clinical ecosystems.

Quantifying health risks from MP-associated pathogens. To characterize the microbial community on MPs, future research should include pathogenicity testing to assess functional processes within the plastisphere. Further investigations are needed to prove that the MP polymer type affects the microbial composition of the microbial community on MPs. The effects of ingested MPs associated with pathogenic microorganisms on different organisms are not well understood. Specifically, the potential health risks of MPs regarding their developmental toxicity is underexplored (Damaj et al., 2024). The lack of data limits quantifying potential health risks from MP-associated pathogens. There is thus a pressing need for larger epidemiological studies to assess the long-term impacts of MP exposure.

Standardization of methods on MP studies. The application of cutting-edge molecular

techniques, particularly next-generation sequencing, represents a transformative advancement in our understanding of biofilms on MPs. These technologies offer unprecedented insights into the complex microbial communities and their functional roles within biofilms, revealing intricate details of microbial diversity, interactions, and ecological impacts. As our ability to analyze these communities with high resolution continues to improve, we can better elucidate the implications of MP pollution for environmental health and ecosystem dynamics. The integration of these advanced methods into future research will be pivotal to developing effective strategies to mitigate the environmental and health risks associated with MP contamination. However, sampling methods, separating, and detecting MPs are notably not standardized yet, and this complicates data comparison across studies (Rede et al., 2023).

Social awareness of MP pollution. Over the past twenty years, researchers have increasingly focused on MP pollution, examining its effects on the environment and human health. Yet, because this type of pollution is often not directly visible or directly related to people's daily experiences, it poses challenges for building awareness and a personal connection to the issue (Mahaliyana & Nugawela, 2024). Effective science communication is, thus, essential for connecting MP research with public awareness globally. Researchers face the challenge of simplifying complex findings to engage the public and highlight individual responsibilities regarding MP pollution. To maximize societal impact, it is crucial to not only study MP biology, their toxicity, and the associated pathogens, but also to understand public attitudes and behaviors towards the issue. Finally, the issue of plastic pollution, macro- or micro- in nature, requires multisectoral participation and interdisciplinary collaboration among scientists, policymakers, and industry leaders (Rede et al., 2023).

Conclusion

This study highlights the emerging and complex ecological risks posed by pathogenic microorganisms associated with MPs. The analysis of literature from 2000 to 2021 revealed that although the number of studies has grown in recent years, the field's limited publication history provides a new foundational information base with significant gaps remaining in the understanding of pathogen transmission mechanisms, their ecological roles, and the resulting human health impacts when associated with MPs. Specifically, only 57 peer-reviewed articles focused on human

pathogenic microorganisms associated with MPs were published across the two-decade period (2000–2021). The initial research efforts are primarily dedicated to collecting and analyzing data, as demonstrated by 41% (23 articles) categorized as Experimental Works, 33% (19 articles) as Review articles, and 26 % (15 articles) characterizing microbial communities on MPs.

The common polymer types hosting these potential pathogens are Polypropylene (PP, 34%) and Polyethylene (PE, 33%), which together accounted for 67% of the polymer associations reported in the 12 studies that met the criteria. This quantitative analysis indicates that the most abundant forms of plastic debris in the environment also serve as the primary substrate for pathogen colonization. The common association of pathogens, particularly species of *Vibrio* and *Pseudomonas*, with PE and PP, two of the most ubiquitous plastic types in the environment, raises concern about their potential to act as disease vectors across aquatic and terrestrial ecosystems.

The findings underscore the need for further studies to examine the influence of polymer type, environmental conditions, and biofilm characteristics on microbial colonization and persistence. Moreover, evidence indicates that MPs may be hotspots for antibiotic resistance genes and horizontal gene transfer, adding another layer of complexity to the risks involved.

While pathogenic microbes dominate current discussions, the presence of beneficial, potentially plastic-degrading microbes on MPs opens opportunities for bioremediation research. However, these avenues are still underexplored. A comprehensive understanding of MP-associated microbial communities, both harmful and beneficial, is crucial for informing environmental management strategies and public health policies. Only through interdisciplinary collaboration can the global community effectively mitigate the risks of MP pollution while harnessing its potential for sustainable solutions.

Authors' Contributions

AAAH conducted the data collection, performed the analysis, and prepared the manuscript. LEER provided overall guidance, led the conceptual refinement, and carried out revisions of the manuscript. HPB provided technical guidance and contributed insights to enhance the manuscript. JVM contributed to the conceptualization phase.

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