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Can plastic pollution drive the emergence and dissemination of novel zoonotic diseases?

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ABSTRACT

As the volume of plastic in the environment increases, so too does human interactions with plastic pollution. Similarly, domestic, feral, and wild animals are increasingly interacting with plastic pollution, highlighting the potential for contamination of plastic wastes with animal faeces, urine, saliva, and blood. Substantial evidence indicates that once in the environment, plastics rapidly become colonised by microbial biofilm (the so-called 'plastisphere), which often includes potentially harmful microbial pathogens (including pathogens that are zoonotic in nature). Climate change, increased urbanisation, and the intensification of agriculture, mean that the three-way interactions between humans, animals, and plastic pollution are becoming more frequent, which is significant as almost 60% of emerging human infectious diseases during the last century have been zoonotic. Here, we critically review the potential for contaminated environmental plastics to facilitate the evolution of novel pathogenic strains of microorganisms, and the subsequent role of plastic pollution in the cyclical dissemination of zoonotic pathogens. As the interactions between humans, animals, and plastic pollution continues to grow, and the volume of plastics entering the environment increases, there is clearly an urgent need to better understand the role of plastic waste in facilitating zoonotic pathogen evolution and dissemination, and the effect this can have on environmental and human health.

1. Introduction

In 2021, almost 400 million tons of plastic were produced (PlasticsEurope, 2022), with concurrently high volumes of plastic pollution released into the environment and an estimated 170 trillion plastic particles (~2.3 million tonnes of plastic) in the oceans (Eriksen et al., 2023). Apart from being unsightly and posing a physical threat to wildlife, there is mounting evidence that environmental plastic pollution can act as a reservoir for viral, bacterial, and eukaryotic human pathogens (Gkoutselis et al., 2021; Metcalf et al., 2022; Moresco et al., 2022; Zhang et al., 2022). Microbial biofilm colonising the surface of plastics is termed the 'plastisphere,' and is comprised of a highly variable, diverse, and genetically distinct community compared to the free-living communities that surround them (Amaral-Zettler et al., 2020). Being in the plastisphere allows human pathogens to survive harsh environmental conditions and retain (and in some cases, enhance) virulence at concentrations capable of causing infection, even following extended periods of desiccation (Ormsby et al., 2023b; Ormsby et al., 2024a, b). Biofilm communities are also well recognised hotspots for horizontal gene transfer (HGT), with intimate interactions between closely (and distantly) related species facilitating the transfer of genetic material, including genes encoding antimicrobial resistance (AMR), and virulence factors (Wang et al., 2021; Yang et al., 2022; Silva et al., 2023).

The durability of plastic provides increased opportunities for colonisation, and in some cases subsequent re-colonisation (Ormsby et al., 2024a), by pathogens from sources of environmental contamination, e.g., wastewater effluent. This may lead to the development of wider risks linked to HGT and thus new and emerging pathogens with enhanced antimicrobial resistance (AMR), more extreme virulence mechanisms, and the acquired ability to colonise new host species. The lightweight and buoyant properties of plastic increase the likelihood of dissemination through the landscape and amplifies the potential for human exposure to contaminated plastics, e.g., at beaches, freshwater and coastal bathing waters, and following urban flooding.

Plastic pollution in a range of environmental matrices (including freshwater, marine and edaphic environments) can be colonised by human pathogens which have zoonotic potential, e.g., *Salmonella*, *Escherichia coli*, *Cryptosporidium*, and rotavirus (Metcalf et al., 2022;

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Zhang et al., 2022; Moresco et al., 2022). It is estimated that 60% of emerging human diseases over the past century have been caused by zoonotic spill-over, including those viruses responsible for major pandemics such as Ebola, influenza, HIV, MERS and SARS-CoV-2 (Saba Villarroel et al., 2023). The rates of zoonotic transmission and the emergence of novel zoonotic pathogens are predicted to increase rapidly in the coming years, exacerbated by climate change, the intensification of agriculture, land-use change, and habitat encroachment (Carlson et al., 2022). Zoonotic transmission can occur through, (1) direct contact between animals and humans; (2) vector-borne transmission from an infected invertebrate; (3) indirect transmission from the consumption of contaminated food or water; and (4) contact with areas containing animal secretions such as saliva, blood, or urine, e.g., urban waste piles. In many low and middle-income countries (LMICs), humans and animals often reside in close proximity, which increases the potential for zoonotic transmission (Hassani and Khan, 2020; Madoshi et al., 2016; Wang et al., 2020). People in LMICs are predicted to be disproportionately affected by zoonotic infections due to poor sanitation and infrastructure, an increasing need to encroach into natural habitats for the provision of food and fuel, limited health-care systems and a high burden of infectious diseases. In addition to this, surveillance data in LMICs has shown that antibiotic resistant bacteria (including those important in human infection) are commonly present in food-producing animals including pigs, chicken, and cattle (Van Boeckel et al., 2019), in part due to the prophylactic use of antimicrobials (Guetiya Wadoum et al., 2016; Hassani and Khan, 2020). A substantial number of these therapeutically used antimicrobial drugs are considered by the World Health Organisation to be 'critically important antimicrobials for human medicine', yet, despite this, it is projected that the use of antimicrobials in livestock systems will continue to rise (Van Boeckel et al., 2017) and thus, promote elevated levels of AMR.

Livestock and wildlife can directly or indirectly interact with environmental plastic waste by grazing or living on or near waste piles, or by drinking from waterbodies contaminated with plastic pollution. Yet, the potential role of plastics as a novel reservoir for zoonotic pathogens, and the hypothetical capacity for the evolution of new and emerging (and drug resistant) zoonoses in the plastisphere remains unknown. In this paper we critically review the available information on the potential for the plastisphere to enable the evolution of novel zoonoses, and to facilitate zoonotic transfer.

1.1. Animal interactions with the plastisphere and the potential for zoonotic transmission

Globally, plastic has become a major constituent of landfills and urban dump sites and is increasing in parallel with both economic development and urbanisation. Waste piles and dump sites, which are common in informal settlements and peri-urban areas of LMICs, contain a range of waste materials such as plastics, glass, wood, and metal, which are routinely salvaged by human waste-pickers (Ferronato and Torretta, 2019; Zolnikov et al., 2021). However, waste piles also include significant volumes of human faeces, either through open defecation or from the dumping of used diapers (Butler et al., 2018; White et al., 2023), whilst the considerable volumes of organic material and food waste attract dogs, pigs, cattle, poultry, and wild animals including birds and rodents, that further contaminate the waste pile with their own saliva, faeces, and urine.

Enteric pathogens in animal hosts can either cause scouring and be discharged in high concentrations in diarrhoea, or can survive in the animal intestine asymptomatically before subsequent shedding into the environment. Animal urine is frequently associated with pathogenic species of *Leptospira* (Haake and Levett, 2015; Cordonin et al., 2020), whilst animal faeces are a significant reservoir for human enteric pathogens (Delahoy et al., 2018). Once in the environment, animal waste can contaminate plastic from direct defecation, or indirectly through surface water or floodwaters contaminated with faeces (Pérez-Guevara et al., 2021). There is mounting evidence that human pathogenic bacteria can interact with plastic waste in both aquatic and terrestrial environments (Metcalf et al., 2022), and persist under a range of fluctuating conditions typical of waste piles, including temperature, humidity, and flooding (Ormsby et al., 2023b; Ormsby et al., 2024a). Non-typhoidal *Salmonella* (NTS) and shiga toxin-producing *E. coli* (STEC) (especially strains of *E. coli* O157) are globally important zoonotic pathogens that commonly infect a wide range of animals (including mammals, birds, reptiles, and insects), and have been shown to persist on plastic waste under conditions simulating an environmental waste pile (Ormsby et al., 2023b; Ormsby et al., 2024a). Therefore, the potential transmission of these pathogens from faecally-contaminated fomites or environments to humans is high, particularly if animals and humans are living in close proximity to each other, as is often the case in many LMIC settings (Penakalapati et al., 2017).

Virus interactions in the plastisphere are poorly understood and compared to bacteria have been significantly under-studied. Human pathogenic viruses can colonise plastic waste in clinical settings; however, it has recently been reported that interaction with the plastisphere can enhance the environmental survival of human pathogenic viruses relative to viruses that are not associated with the plastisphere (Moresco et al., 2022). While many zoonotic viruses currently require either direct contact or aerosolisation for transmission to a new host, some viruses are transmissible through the faecal-oral route, e.g., hepatitis A, hepatitis E, rotavirus (Reed, 2018; Lanrewaju et al., 2022). Following sewage and wastewater spills, open defecation, agricultural run-off, and flooding events, viruses are the most likely pathogens to encounter and colonise environmental plastic waste (Moresco et al., 2021) and present the greatest risk for zoonotic transmission. However, human-specific pathogens that do not currently represent a zoonotic transmission risk, will also associate with the plastisphere, and could come into direct contact with a potential animal host, e.g., through consumption of plastics in water of food. Such pathogens may adapt to survive in a new host, either through acquisition of new genetic material (e.g., from associations within the plastisphere), undergoing beneficial mutations, or by taking over the hosts cellular metabolism (Metcalf et al., 2024). The establishment of a viral pathogen in a new animal host then has the potential to lead to the evolution of more virulent strains or strains more capable of avoiding the host immune response, leading to greater risks of infection and transmission.

It is not just through environmental contamination that plastic waste in the environment can become colonised by bacterial and viral pathogens. Animals can consume plastic particles, including livestock feeding on plastic contaminated foods (Wu et al., 2021) or via interactions with waste piles, which will contain plastics colonised by plastisphere biofilm (Fig. 1). Ingested plastic will encounter autochthonous microbial communities that will also adhere to and associate with the environmental plastisphere community. Subsequent defecation by the animal will therefore contain plastics colonised by pathogens capable of survival in the animal host, e.g., *E. coli* O157, which will subsequently be disseminated in the environment with enhanced survival facilitated by colonisation of the plastisphere (Ormsby et al., 2023b).

The rate of zoonotic disease emergence from wildlife reservoirs has increased over recent decades, with a correlation between emerging pandemics, increasing contact between humans and animals, and changing environmental conditions (Wu et al., 2021). Wild and feral animals consume and defecate on waste piles within peri-urban environments, likely resulting in the repeated passage of faecal-orally transmitted organisms through the mammalian/avian digestive tract, and the environment. This will facilitate selection of pathogenic variants better adapted for a combination of both *in vivo* and environmental survival. Repeated passage through the animal digestive tract can result in the selection of fitter variants better adapted for *in vivo* survival (Ormsby et al., 2023a), while adaption to environmental conditions such as temperature and pH have been demonstrated for zoonotic bacterial pathogens including *E. coli* and *Salmonella* spp. (Keerthirathne

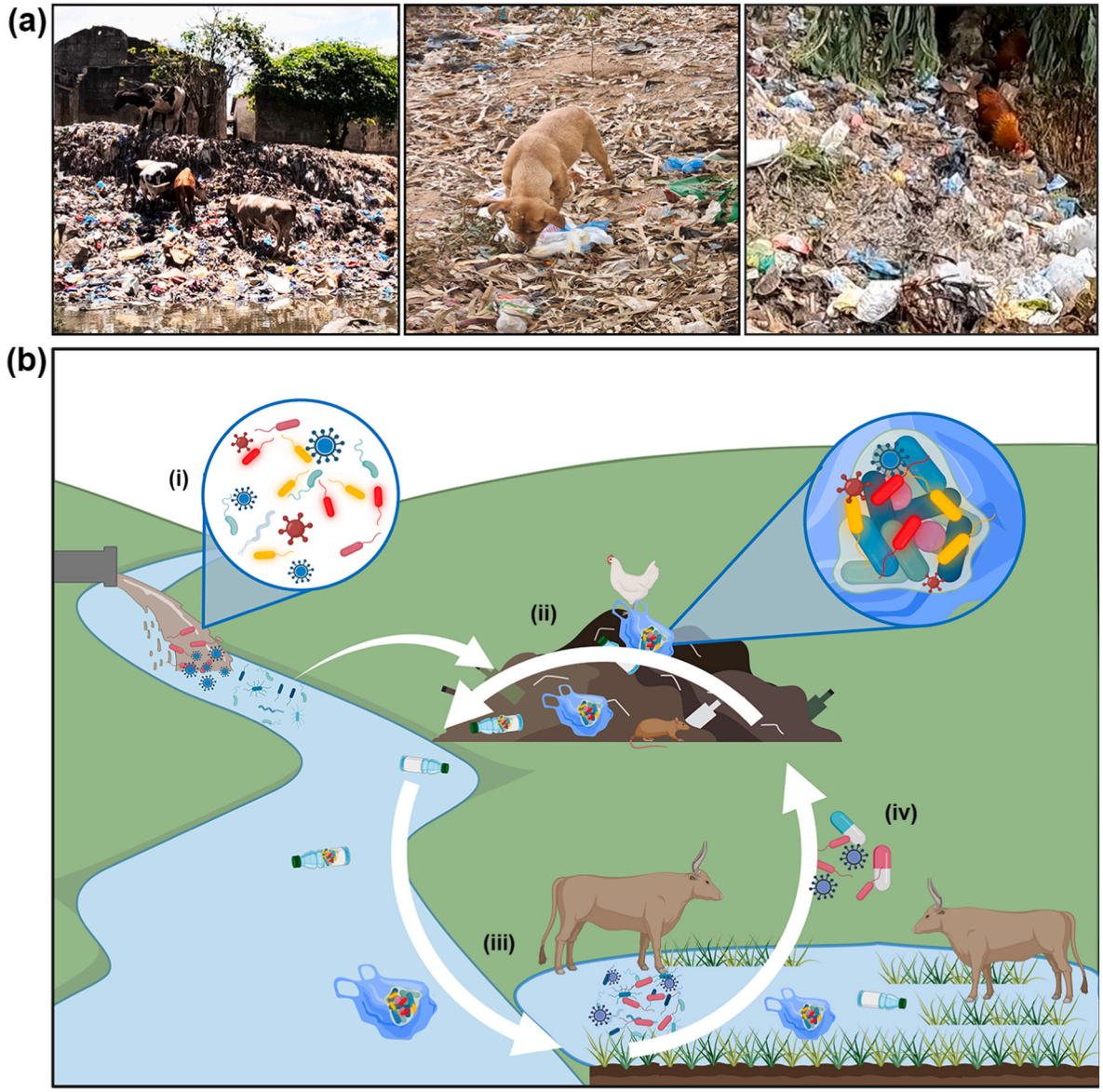

Fig. 1. The role of the plastisphere in the dissemination of pathogens between waste piles and animals. **(a)** Animals often frequent waste piles and interact with plastic waste. **(b)** Wastewater effluent can be a significant source of human pathogens and microplastics (i); Waste piles, often containing large volumes of plastic, can become contaminated by foraging animals (ii); Flooding events lead to the transport and dissemination of plastics and plastisphere-associated pathogens into the environment (iii); Agricultural run-off can contain antibiotics and animal/zoonotic pathogens, and facilitate the transmission of plastisphere-associated pathogens containing genes for AMR (iv). Arrows indicate the potential movement of pathogens between settings. Figure was generated using BioRender (https://biorender.com/).

et al., 2016; Riehle, 2001). The rate of viral spillover from animal reservoirs to humans or livestock naturally fluctuates with levels of transmission and is dependent on a variety of environmental and host-specific factors (Plowright et al., 2016). Viruses such as Ebola or Marburg, fluctuate between host populations and environmental reservoirs, and the volume of plastic pollution now in the environment could be providing a platform for increased spillover potential by increasing exposure and transmission pathways. Thus, these hypothetical cyclical transfers between host and plastic waste (as an environmental reservoir) could result in pathogen variants better adapted for *in vivo* and environmental survival, which together with the protective properties of the plastisphere, and the persistent, lightweight, and buoyant properties of plastic polymers could enable these pathogens to be better disseminated throughout the landscape.

Exemplified by the recent covid-19 outbreak, viral pathogens represent the greatest current risk for zoonotic spillover and infection of human hosts. While humans are constantly exposed to animal viruses, those that can successfully jump from an animal host to humans are rare. However, when this does occur, the rapid evolution and transmission of viruses means the outcome can lead to significant epidemic or even pandemic proportions. Transmission of zoonotic viruses from animals to humans can occur via direct or indirect contact, requiring a point of contact between the animal and human, either directly or through an environmental source (Nandi and Allen, 2021). For example, animals interacting with plastics in the environment could lead to human exposure to animal viruses in the plastisphere and the subsequent emergence of novel zoonoses.

The close genetic relatedness between some animal and human viruses may further encourage this transition. Where novel human norovirus genotypes and variants are emerging from is unclear, but the close genetic relatedness between some animal and human norovirus strains has led to the hypothesis that noroviruses may not be host

restricted and might be able to jump the species barrier. Although there are no reports of human infections from animal noroviruses, zoonotic potential of norovirus transmission from other mammals, especially those farmed for human consumption, to humans via the food chain cannot be neglected (Villabruna et al., 2019). Group A Rotavirus (RVA) shows vast diversity, and a variety of human strains share genetic and antigenic features with animal origin Rotavirus A strains. This finding suggests that interspecies transmission is an important mechanism of rotavirus evolution and contributes to the diversity of human RVA strains (Doro et al., 2015). The high concentrations of microplastics routinely released in both wastewater and sewage effluent, remain a major source of microplastics entering the aquatic environment, with an annual input estimated at 10^{15} particles (Lares et al., 2018; Kelly et al., 2021; Pittura et al., 2021). Sewage and wastewater can also contain a high concentration of human enteric viruses, including both rotavirus and norovirus, which are routinely shed in high concentrations in the faeces of infected individuals (Farkas et al., 2018; Prado et al., 2019), and provides a significant opportunity for human viruses to interact with microplastics in wastewater and the receiving surface water.

1.2. *Potential for plastisphere-driven bacterial evolution and the emergence of novel pathogens*

Livestock are a valuable commodity in many countries, and according to the FAO, contribute nearly 40% and 20% of total annual agricultural output in developed and developing countries respectively (FAO, 2023). Antimicrobials are commonly used to maintain healthy livestock populations; however, resistance to many of our 'last resort' antibiotics correlates with their intensive use in agriculture (Ikhimiukor et al., 2022). This includes the emergence of global colistin resistance (Liu et al., 2016; Sun et al., 2018; Wang, 2022), and the identification of tetracycline resistance genes in both animals and humans (He et al., 2019; Sun et al., 2019). Antimicrobials used in arable and livestock agriculture frequently run off into soil and water ecosystems, where there is extensive opportunity for contact with plastic and microplastic pollution (and the associated plastisphere) (Manyi-Loh et al., 2018). Over time, as the plastisphere matures and recruits more microorganisms (including human pathogens), the exchange, or uptake, of genetic material (e.g., virulence factors and resistance to antimicrobials) within the biofilm can lead to the evolution of novel gene combinations (Metcalf et al., 2024).

Horizontal gene transfer (HGT) is an important process for driving novel genetic variation and adaptive traits and can play a critical role in adaptation and organismal evolution by overcoming the disadvantages of asexual reproduction for both prokaryotes and eukaryotes (Woods et al., 2020). In bacteria, HGT can drive rapid adaptation to novel stresses, and contribute to the evolution of traits like antibiotic resistance (Carr et al., 2021; Acar Kirit et al., 2022). Mechanisms through which HGT can occur within bacteria, include conjugation, transformation, transduction, and transfer of genetic material via membrane

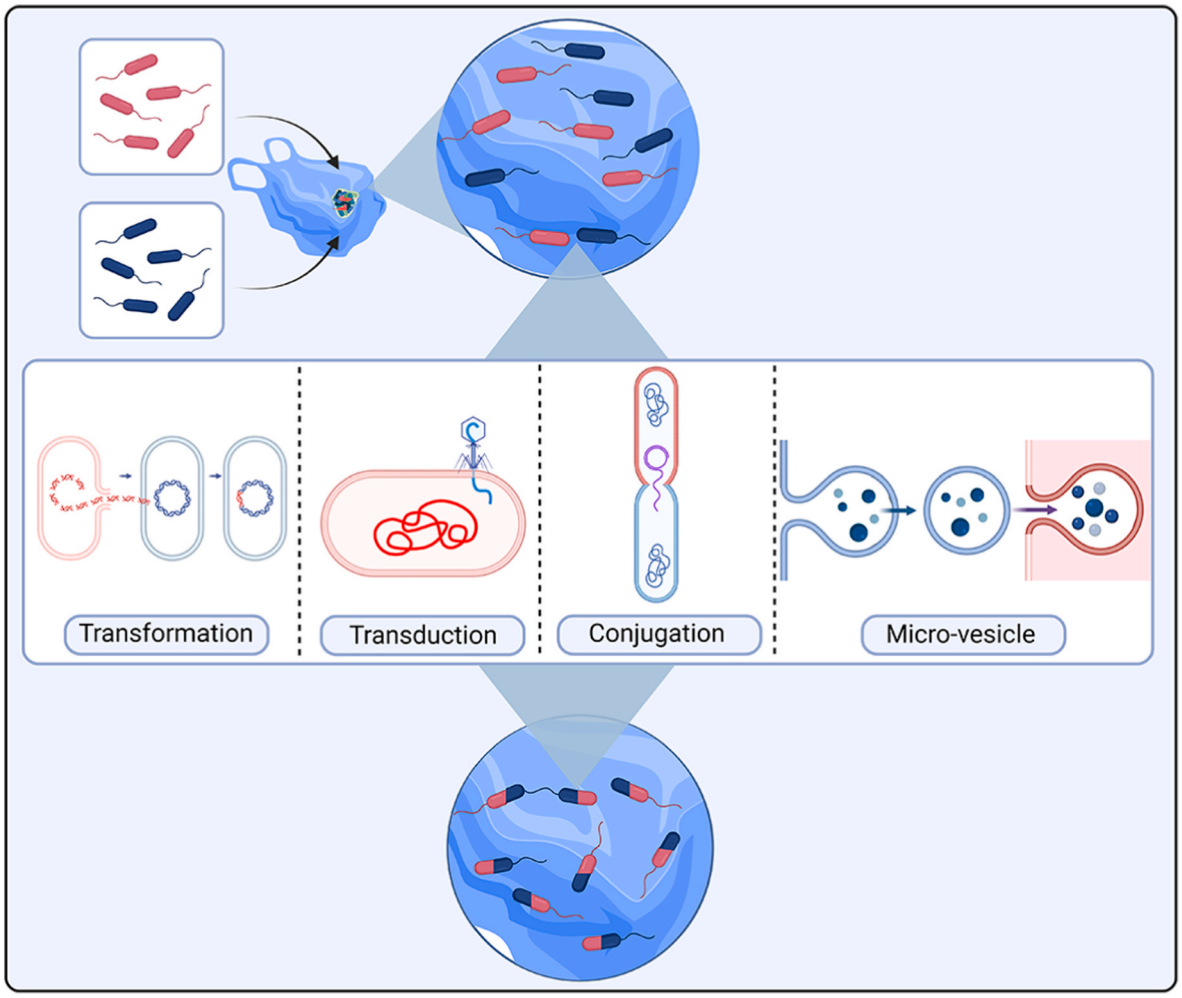

Fig. 2. HGT mechanisms that can lead to the potential emergence of novel bacterial pathogens in the plastisphere. Genetic material can be exchanged between two different bacterial species (depicted here as red and blue) when they are found together in the plastisphere, and as the biofilm matures and is exposed to environmental stressors exchanges of genetic material can occur between cells. Figure was generated using BioRender (https://biorender.com/).

vesicles (Fig. 2). Conjugation involves direct contact between donor and recipient bacteria, after which mobile genetic elements (MGEs; including plasmids) are transferred via conjugative pili (Sørensen et al., 2005); transformation involves the direct uptake of free DNA by bacteria from the environment and the acquisition of the corresponding genetic traits (Arnold et al., 2022); transduction occurs from DNA transfer through phage vector infection (Chen et al., 2018); and membrane vesicles are capable of transporting DNA material between bacteria (Ghanam et al., 2022).

The plastisphere is a 'hotspot' for the sharing of genetic information, with a higher frequency of plasmid transfer between plastic-associated bacteria compared to free-living bacteria (Arias-Andres et al., 2018). Microorganisms in the plastisphere will experience a range of environmental stressors (many of which will be amplified by climate change), including extremes of temperature and intense UV exposure, desiccation, nutrient limitation, antibiotic pressure, and exposure to fluctuating salinities and pH. Environmental pressures induce stress responses in bacteria (e.g., the general SOS response (Hocquet et al., 2012)), and in the plastisphere the stress responses to the changing environmental conditions may also influence mechanisms of HGT. For example, induction of responses to either heat shock or cold shock can induce an increase in the rate of genetic recombination and HGT, although lower temperatures can decrease the rate of conjugation in bacteria (Pallares-Vega et al., 2021). Whilst the plastisphere could provide protection against the most extreme temperature fluctuations, temperature can also impact the density, composition, and stability of the biofilm, and in *Salmonella* can increase expression of genes associated with virulence (Badie et al., 2021; Roy et al., 2021). UV irradiation activates the bacterial SOS response, which when coupled with elevated temperatures, can lead to the activation of lambda prophages (Krishna et al., 2007; Patel et al., 2010). UV irradiation in combination with high temperatures accelerates the movement of Shiga toxin (Stx) genes between pathogenic Enterohaemorrhagic *E. coli* (EHEC) and non-pathogenic *E. coli* (Yue et al., 2012). It is expected that extremes of temperature will continue to increase (together with increasing UV exposure) in the future due to climate change, and it is likely that such transfer events may become more common.

Challenging biofilms containing *E. coli* and *P. aeruginosa*, with the antimicrobial compounds tetracycline and cephradine can increase plasmid transfer (Salcedo et al., 2015), while exposure to sublethal concentrations of antibiotics can induce the bacterial SOS response (Kohanski et al., 2010; Gullberg et al., 2011). Hospital discharge, wastewater treatment plants (WWTPs), receiving waters, and agricultural soils are all sinks for pharmaceuticals, including antibiotics, which can become incorporated into biofilms, including the plastisphere (Wang et al., 2022; Martínez-Campos et al., 2023). Sub-inhibitory concentrations of β-lactams and fluoroquinolones can induce SOS responses and increase mutation rates in bacteria including, e.g., *E. coli* and *P. aeruginosa* (Andersson and Hughes, 2014). The SOS response can induce the expression of an integrase that induces integrons, and activate virulence genes and genes encoding antibiotic resistance (Kreuzer, 2013). Antimicrobial resistance genes are commonly identified in the plastisphere in soil, freshwater and marine environments (Bourdonnais et al., 2022; Singh et al., 2022; Zhu et al., 2022); as plastics can persist in the environment for long periods of time there is the potential for colonisation and acquisition of genetic material by many generations of a wide diversity of pathogens in this environmental hotspot of microbial genetic exchange.

In the environment, pathogens shed by humans, livestock, and wild animals will all encounter plastic pollution, with the potential to become part of the same plastisphere community. Such close contact within this novel microbial niche provides the opportunity for genetic exchange and an increased risk of host-specific gene transfer between unrelated pathogens. This in turn may lead to the evolution of novel strains of pathogens which are able to colonise new hosts. Should this be the case for a pathogen such as *V. cholerae*, this could have devastating implications for transmission and could result in outbreaks of disease out with the typical cholera season or with increased virulence or transmissibility.

Although viruses require a host to replicate, there is still evidence that they can evolve and adapt to changing environmental conditions, as observed with enteroviruses that are capable of generating thermotolerance. Experimental analysis has revealed that NaCl can enhance the thermostability of enteroviruses through increasing the capsid-protein interactions (Meister et al., 2020); and that exposure to a high temperature (30 °C) can result in increased thermotolerance, compared to strains adapted to a lower temperature (10 °C), which are rapidly inactivated at higher temperatures (Carratalà et al., 2020). Similarly, human echovirus 11 has generated a form of UV resistance, resulting in reduced UV-C inactivation rates and an associated increased resistance to ribavirin, an antiviral drug which interferes with viral replication in a similar manner to UV-C exposure (Carratalà et al., 2017). The plastisphere can provide protection against environmental factors such as UV; therefore, the protection afforded to viruses that associate with the plastisphere may encourage the development of similar resistance mechanisms.

The plastisphere could be providing a favourable habitat for the evolution and emergence of pathogenic strains with novel AMR and virulence phenotypes. However, this hypothetical process now needs testing, for example, through coinfection studies on different abiotic materials, under varying environmental parameters, followed by subsequent *in vitro* cell line infection studies to examine rates at which pathogens can share information within the plastisphere, and the likelihood that emerging pathogens with zoonotic potential will appear.

2. Conclusions

Data supporting the role of the plastisphere for the survival and dissemination of human pathogens is rapidly increasing, which is important for a more complete understanding of the links between plastic waste and human health. However, whether the plastisphere can facilitate the evolution of novel zoonotic pathogens capable of adapting to changing environments is poorly understood. The first step needed to quantify this potential is an understanding of the rates of genetic transfer in the plastisphere between microorganisms with zoonotic potential, particularly when exposed to climate change stressors, including increasing temperatures, salinity, UV exposure, flooding and drought scenarios.

Pivotal to combating the dissemination of plastisphere-associated pathogens and the potential for novel pathogen emergence within this niche, is the need to increase education and understanding of the risks associated with plastic waste. With increasing reports of plastic pollution and contaminated plastic waste being found in locations where human exposure can be high, there is a heightened potential for the spread of disease to the human population. Government and policy makers must prioritise environmental plastic waste as a human health priority to truly understand the risks associated with it. This should include improving education on the risks associated with waste piles and through implementing more stringent animal husbandry practices to reduce the interaction between animal and human waste.

CRediT authorship contribution statement

Michael J. Ormsby: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Luke Woodford:** Writing – review & editing, Conceptualization. **Richard S. Quilliam:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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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