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Rapid colonisation of environmental plastic waste by pathogenic bacteria drives adaptive phenotypic changes

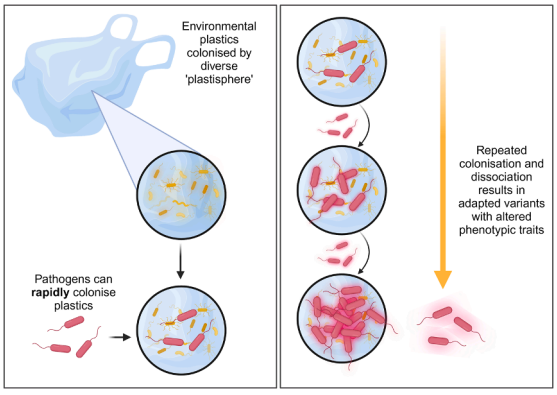
Michael J. Ormsby *, Luke Woodford, Rosie Fellows, Hannah L. White, Richard S. Quilliam

Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA. UK

HIGHLIGHTS

- Plastic can be rapidly colonised with potentially pathogenic bacteria.
- Pathogenic bacteria become adapted to the plastisphere following repeated exposure.
- Plastisphere adaptation can result in concomitant increases in biofilm formation and antibiotic tolerance.
- The co-pollutant risk of plastics and pathogens poses a heightened human health risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial biofilms on environmental plastic pollution can serve as a reservoir for both pathogenic and commensal bacteria. Associating with this 'plastisphere', provides a mechanism for the wider dissemination of pathogens within the environment and a greater potential for human exposure. For pathogens to bind to environmental plastic waste they need to be in close contact with it; therefore, understanding how rapidly pathogens can bind to plastics and the temporal colonisation dynamics of the continual cycling between the plastisphere and the environment are important factors for quantifying the persistence of human pathogens. Using simulated environmental conditions, we demonstrate that pathogenic *E. coli* O157 can rapidly colonise plastics (within 30 min) and persist for extended periods (at least 21 days), at concentrations sufficient to cause human infection. Importantly, repeated colonisation and dissociation cycles of *E. coli* O157 from the plastisphere leads to an enhanced capacity for persistence and the emergence of variants with increased virulence traits, including improved biofilm formation and antibiotic tolerance. This phenotypic adaptation to repeated colonisation of environmental plastic surfaces could be selecting for more persistent and virulent strains of pathogens, and hence increase the co-pollutant risks associated with plastic pollution.

* Corresponding author.
E-mail address: Michael.ormsby1@stir.ac.uk (M.J. Ormsby).

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1. Introduction

It is estimated that over 220 million tonnes of plastic waste will be created in 2024, with almost 70 million tonnes ending up in the environment [1], which represents an increase of over 7 % since 2021. However, the recalcitrant and durable properties of plastic polymers mean they are capable of persisting for hundreds of years in the environment, which is in stark contrast to the persistence profiles of natural materials such as fabrics, e.g., linen (< 2 weeks), cotton (< 5 months), and silk (< 4 years) [2]. Once in the environment, plastic rapidly becomes colonised by biofilm comprising a diverse microbial community, which includes both eukaryotic and prokaryotic species [3]. Collectively, this community is known as the plastisphere [4] and is often distinctly different to that of the surrounding environment, with evidence suggesting that the plastisphere can selectively enrich certain organisms [5-7].

It is now well established that human pathogenic viral, fungal, and bacterial species can colonise and persist on the surfaces of plastic waste [8-10]. Such pathogens are entering the environment with increasing frequency, e.g., through sewage and wastewater discharge [11], and beyond simply colonising and persisting on plastic waste, human pathogenic bacteria can retain (or even enhance) their virulence or pathogenicity following interaction with the plastisphere, suggesting the potential for the selection, or evolution, of more virulent variants [12, 13]. Importantly, colonised plastic pollution can be widely disseminated throughout the landscape, with pathogens capable of subsequent dissociation from the plastisphere and replication to infectious levels (e.g., *Vibrio cholerae*; [14]).

Plastisphere research has progressed rapidly in recent years, although many fundamental questions regarding pathogen colonisation dynamics remain, e.g., the rate at which pathogens colonise plastics under environmental conditions. Initial bacterial attachment to surfaces can occur quite rapidly (e.g., [15,16]), although within the first few minutes, the association is often weak and reversible being based on hydrodynamic and electrostatic interactions [17]. The majority of plastisphere studies begin their quantification of plastic colonisation after the initial 24 h (e.g., [18-20]); however, by this time, secondary bacterial attachment mechanisms involving interactions between the hydrophobic regions of the outer cell wall of the bacterium and the plastic surface, together with the expression of many bacterial structures involved in adhesion, including lipopolysaccharide, pili, fimbriae, and extracellular polymeric substances (EPS) will already have occurred.

While pathogens can readily dissociate from the plastisphere and replicate to levels capable of causing human infection, the adaptative ability of such pathogens to recolonise plastic waste following their dissociation remains unknown. Adaptation to plastics would provide a competitive advantage over other species and may be an important factor in the environmental survival of some pathogens [21,22]. Previous work has shown that *V. cholerae* can dissociate from the plastisphere, replicate in water, and subsequently recolonise plastic [14]; and that in soil, *Salmonella* Typhimurium can dissociate from the plastisphere, and via transfer through soil leachate, colonise new particles of plastics [23]. Understanding whether this initial association with plastic could 'prime' pathogens for subsequent colonisation of plastic pollutants is important when considering the cyclical potential for pathogens to transition from the environment to plastic, and following subsequent dissemination, back into the environment. Importantly, the colonisation and recolonisation of environmental plastics presents opportunities for the transfer of genetic material, including the exchange of plasmids between species, and the uptake of exogenous plasmids and DNA from previous colonisers. While there is evidence that biofilms are hotspots for such horizontal gene transfer (HGT) events [24-26], direct confirmation that this occurs in the plastisphere, or indeed that pathogenic organisms are gaining additional traits such as antimicrobial resistance (AMR), is lacking.

Plastic pollution is often rapidly transported through aquatic systems, e.g., rivers and coastal environments, and subsequently, there are limited opportunities for plastics and pathogens to come in contact with each other. Therefore, an understanding of the temporal colonisation dynamics of plastics and pathogens is needed to more fully understand the context-specific risks associated with environmental plastics. In environmental settings, many plastics will already be colonised by a diverse biofilm matrix prior to pathogen exposure, so examining the relative potential for human pathogens to bind to the surface of the plastic, or associate with the plastisphere community as part of a secondary biofilm, will further advance our understanding of plastic-pathogen dynamics. Therefore, in this study, we aimed to: (1) determine the rate at which plastics and organic material (banana leaf) become colonised by an environmentally relevant pathogenic strain of *E. coli* O157 under simulated environmental conditions; (2) determine if *E. coli* O157 could become adapted to the plastisphere with enhanced colonisation potential over subsequent generations; and (3) determine if this adaptation influenced phenotypic traits including biofilm formation and AMR.

2. Materials and methods

2.1. Bacterial strain and growth conditions

A chromosomally *lux*-marked (Tn5 *luxCDABE*) strain of nontoxigenic *Escherichia coli* O157:H7 (encoding Kanamycin resistance; [27]) was maintained in Luria-Bertani (LB) broth or on agar (Invitrogen, UK) supplemented with Kanamycin (50 μg/ml), and grown at 37 °C. Stock cultures were kept in 40 % (vol/vol) glycerol at −80 °C.

2.2. Formation of natural biofilms on surfaces of polyethylene and banana leaf

Blue high-density polyethylene (PE; 20 μm, Thali outlet, Leeds, UK) and sections of banana leaf (BL; Lakshmi stores, UK) were housed in bespoke stainless-steel frames (Fig. 1; [28]). Half of the frames were used with virgin (non-biofilm) material, and half of the frames were colonised by natural biofilm generated by submerging them in river water collected from the Allan Water (Bridge of Allan, Scotland, UK) supplemented with a trace metal solution ($CuCl_2.2H_2O$, 15 mg/L; $NiCl_2$. H2O, 25 mg/L; $Na_2MoO_4.2H_2O$, 25 mg/L; $ZnCl_2$, 70 mg/L; $MnCl_2.4H2O$, 100 mg/L; $CoCl_2.6H2O$, 120 mg/L; $FeCl_3$, 4 g/L; EDTA, 2 g/L; HCl [25 %], 6.5 ml/L) [29] for 96 h at room temperature (ca. 18–21 °C), and continuously aerated using an air pump (Fig. 1). The conductivity (145.9 ± 3.5 μS), pH (7.6 ± 0.1), and turbidity (4.6 ± 0.5 NTU) of the river water were measured using a portable probe (Combo pH and EC, Hanna Instruments Ltd., UK) and a HI-88703–02 Bench Top Turbidity Meter (Hanna Instruments Ltd., UK). The river water was screened to ensure the absence of kanamycin-resistant bacteria, prior to use.

2.3. Washing efficiency

To ensure that only those cells of *E. coli* O157 that were strongly associated with the plastisphere during the rate of colonisation experiment were enumerated (Fig. 1a), it was essential to determine the minimum number of PBS washes necessary to dislodge loosely attached material from the plastisphere biofilm, without disrupting the biofilm itself. Washing efficiency tests were carried out to ensure that only *E. coli* O157 cells that were strongly adhered to the plastisphere were enumerated. Briefly, 300 μl of *E. coli* O157 (1.05E+08 ± 3.97E+07) was inoculated onto squares of the virgin PE and BL and the squares containing the natural biofilm, before incubation at 20 °C for 2 h. Each square of material was subsequently rinsed with 0, 1, 2, 3, 5, 7, 10 or 15 ml of PBS in iterative aliquots of 1 ml, and the PBS discarded. Next, 300 μl of PBS was added to each square, before the biofilm was disrupted by gentle scraping. Strongly adhering cells of *E. coli* O157 were enumerated by plating on LB with kanamycin (50 μg/ml) and incubated

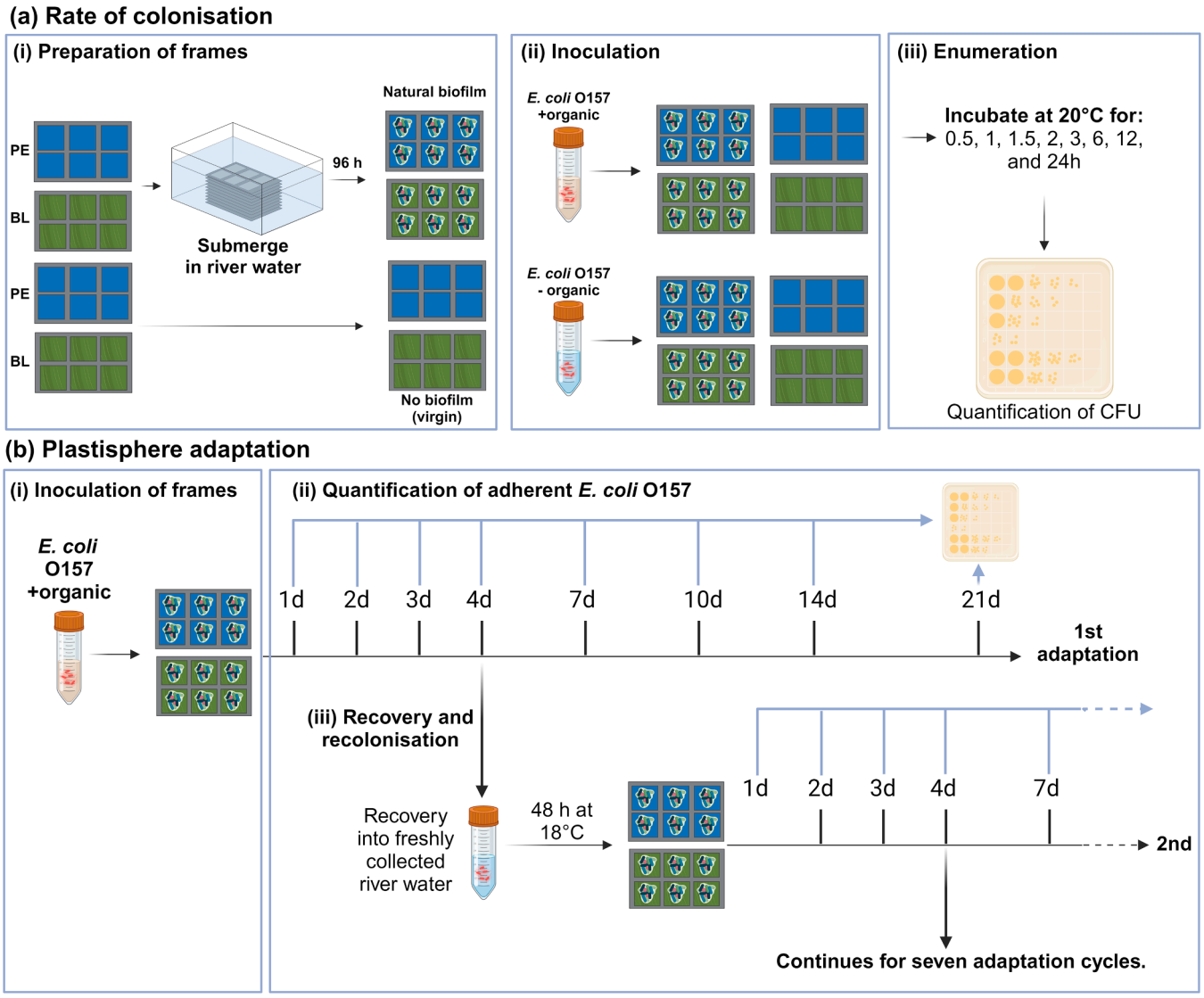

Fig. 1. Schematic overview of workflow for examining the colonisation and recolonisation rate of *E. coli* O157 on polyethylene (PE) and banana leaves (BL). (a) Rate of colonisation. **(b)** Plastisphere adaptation, at the 4d timepoint, adherent *E. coli* O157 was scraped off and used as an inoculum for a new set of frames. This process was repeated for seven cycles, with six biological replicates. Figure generated using BioRender (https://biorender.com/).

at 37 °C for 24 h. Three independent biological replicates were performed at each timepoint.

2.4. Rate of E. coli O157 colonisation

A suspension of *E. coli* O157 was prepared by back dilution of an overnight culture into fresh LB broth and grown at 37 °C until an $OD_{600\ nm}$ of 0.5 was reached. Cultures were pelleted by centrifugation and resuspended in either river water, or a faecal suspension of river water containing autoclaved faeces (10 mg/ml), and 300 µl pipetted onto the surface of each material (2.90E+08 ± 8.03E+07). All frames were incubated at 20 °C. At designated time points (0.5, 1, 1.5, 2, 3, 4, 6, 12, and 24 h), squares of material were excised from the frame and gently rinsed with three washes of sterile PBS (each 1 ml) to remove loosely attached cells. The biofilm was removed by adding 350 µl of PBS to each square and gently scraped with a sterile loop. *E. coli* O157 was quantified by serial dilution and plating onto LB supplemented with Kanamycin (50 µg/ml), incubated at 37 °C for 24 h and colony forming units (CFUs) enumerated. Three independent biological replicates were performed at each timepoint.

2.5. Adaptation of E. coli O157 following repeat exposure to the plastisphere

The ability of *E. coli* O157 to adapt to the plastisphere was examined through repeated colonisation and recovery from independent sets of frames containing PE and BL, with or without natural biofilms (Fig. 1b). Six independent biological replicates of *E. coli* O157 (2.0E+08 ± 6.32E+07) were added to individual squares of each material on an initial set of frames and then placed into a sealed box and incubated in the dark at 20 °C. At designated time points (1, 2, 3, 4, 7, 10, 14, and 21 days), 300 µl of PBS was added to each square of material, and left for 15 min to loosen the biofilm, followed by gentle scraping. Viable *E. coli* O157 were quantified by serial dilution and plating onto LB supplemented with Kanamycin (50 µg/ml) and incubated at 37 °C for 24 h. At day 4, the scraped material was resuspended in fresh river water for 48 h at 20 °C to simulate dissociation from the plastisphere. This suspension was then used as the inoculum for a second set of frames (which had been colonised by biofilm with freshly collected river water, as described above). This process was continued for seven cycles, with a stock being made of each sample at the 21-day time point, as described previously. PCR was performed to confirm the identity of the *E. coli*

O157 strain on each of the 21-day samples, using primers for *E. coli* O157 [30] (data not shown). Each week, fresh river water was collected and used for biofilm generation on a new set of frames, and the conductivity, pH, and turbidity were measured for each cycle (Supplementary Fig. S2).

2.6. Biofilm assay

Biofilms were enumerated using the crystal violet method [14,31]. Cultures of *E. coli* O157 directly recovered from the squares at each timepoint were stored in glycerol, and subsequently used in all biofilm assays rather than first regrowing them in LB to avoid reversion to a wild-type phenotype. The wild-type control *E. coli* O157 was also treated in the same way. Briefly, 100 µl of glycerol stock was diluted in 10 ml PBS, thoroughly mixed, and 100 µl of the resultant suspension added to each well of a 96-well plate (Greiner, UK), covered with a plate seal (ThermoFisher, UK), and placed inside a sealed plastic box containing damp tissue paper to maintain humidity. Boxes were incubated at 20 °C for 48 h. Planktonic cells were gently removed by washing the wells with PBS before letting the plate air-dry. To each well, 100 µl of 0.1 % crystal violet was added and left for 20 min before gentle rinsing with dH_2O. Next, 200 µl of 30 % acetic acid was added to each well, and the absorbance was measured at $OD_{550\ nm}$. Six independent biological replicates were used, and results are presented as the mean ± SEM.

2.7. Minimum inhibitory concentration (MIC)

The resistance and sensitivity to antibiotics was tested against, erythromycin (11.7 µg/ml to 6 mg/ml in two-fold serial dilutions); ampicillin (3.9 µg/ml to 2 mg/ml); chloramphenicol (0.8 µg/ml to 400 µg/ml); gentamycin (0.8 µg/ml to 400 µg/ml); tetracycline (0.4 µg/ml to 200 µg/ml); streptomycin (3.9 µg/ml to 2 mg/ml); and tobramycin (0.8 µg/ml to 400 µg/ml). Cultures of *E. coli* O157 directly recovered from the squares at each timepoint were stored in glycerol, and subsequently used in all MIC tests rather than regrowing them in LB to avoid reversion to a wild-type phenotype. Briefly, 100 µl of the glycerol stock was diluted in 10 ml PBS, thoroughly mixed and 10 µl of the resultant suspension added to each well of a 96-well plate, which contained decreasing concentrations of each antibiotic. Cultures were grown in LB containing no antibiotic as a positive control and LB containing no bacteria was included as a negative control. Samples were incubated statically overnight, at 37 °C. Growth was measured using $OD_{600\ nm}$. Six independent biological replicates were used, and results are presented as the mean ± standard error.

2.8. Statistical analyses

Generalised linear models with logged response variables and listed independent variables were used for statistical analysis in Fig. S1 and Fig. 2. In Fig. S1, the natural log of CFU was used as a dependent variable

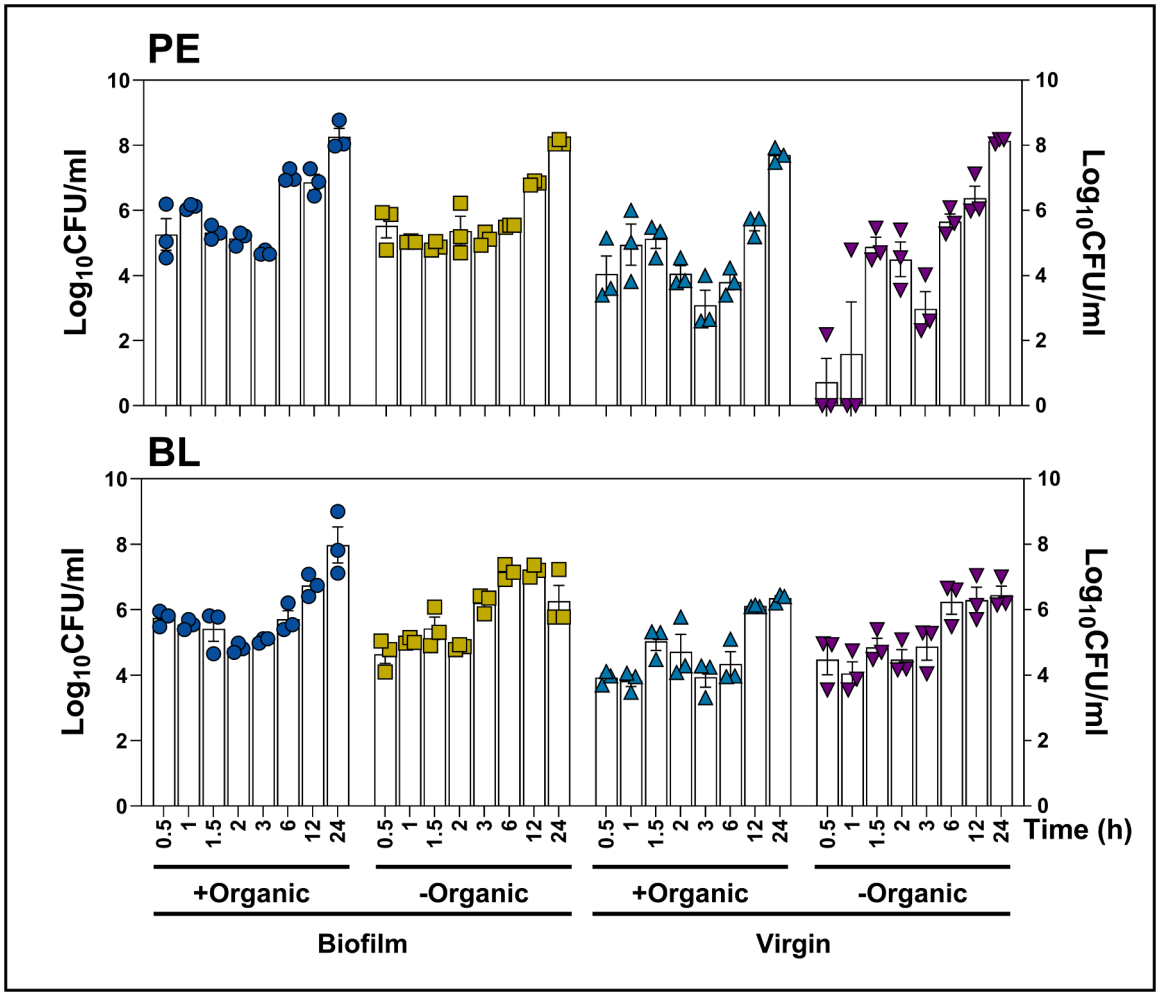

Fig. 2. Colonisation rate of *E. coli* O157 on PE and BL with or without natural biofilm and with or without organic material in the inoculum. BL and PE were either colonised with a biofilm (Biofilm) or no (Virgin) prior to inoculum with *E. coli* O157. The inoculum either contained suspended organic (faecal) material (+) or did not (-). Symbols represent each of three biological replicates, and bars represent the mean (± SE).

and the number of washes, surface type, and the presence or absence of biofilm as independent variables. In Fig. 2, the natural log of CFU was used as a dependent variable and time, surface type, presence of biofilm, and presence of organic material in the inoculum as independent variables; interactions between time and the three other independent variables were also considered in the model. The full model was conducted using R (R [32]) and in-house code. To determine differences in the persistence of *E. coli* O157 that was repeatedly cultured on PE and BL, Brown-Forsythe and Welch ANOVA tests were performed; and to determine differences in biofilm formation of *E. coli* O157 following repeated culture on PE and BL, a two-way ANOVA with Holm-Šídák multiple comparisons post-test was performed. In both cases, analyses were conducted using Prism Software (Version 10.3.2, GraphPad). *P* values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Washing efficiency

A general linear regression model was used to determine how surface type, biofilm presence, and the number of PBS washes influenced the recovery of *E. coli* O157 from the surface of PE and BL (Fig. S1). The number of washes had a significant effect on the concentration of *E. coli* O157 recovered from PE and BL, with significantly less *E. coli* O157 recovered as the number of PBS washes increased from zero washes, to fifteen ($P < 0.001$). Significantly more *E. coli* O157 remained associated with the PE and BL following PBS washes when there was a biofilm present on the material surface, relative to when no biofilm was present ($P < 0.001$). The surface type (PE or BL) did not significantly affect the amount of *E. coli* O157 recovered following PBS washes, with no significant differences observed in the amount of adherent *E. coli* O157 between PE and BL following each wash. No significant differences were observed in the recovery of *E. coli* O157 between three and 15 PBS washes for either PE or BL, therefore samples were routinely washed with PBS three times prior to scraping and enumeration in subsequent experiments.

3.2. Rate of colonisation

A general linear regression model was used to determine how surface type, biofilm presence, and the presence of organic material in the inoculum influenced the rate of colonisation of *E. coli* O157 on PE and BL (Fig. 2). In all cases, the amount of *E. coli* O157 adhering to PE and BL (with or without biofilm; and with or without organic material added to the inoculum) significantly increased over time ($P < 0.001$); however, no significant differences were observed in the overall rate of colonisation between BE and PL. While the presence of biofilm on both PE and BL prior to the addition of *E. coli* O157 significantly increased the rate of colonisation relative to when biofilm was absent ($P < 0.05$), the addition of organic (faecal) material to the *E. coli* O157 inoculum did not significantly affect the rate of colonisation. However, there was a significant two-way interaction between surface type and time (interaction coefficient = 0.352; $P < 0.001$); and surface type and the presence of organic material (interaction coefficient = −0.255; $P < 0.05$).

3.3. Repeated plastisphere colonisation leads to plastisphere adaptation

Repeated colonisation of environmental plastisphere biofilms by *E. coli* O157 resulted in a greater capacity for persistence compared to non-adapted strains of *E. coli* O157 (Fig. 3). Seven cycles of plastisphere colonisation, each lasting 4 days, increased the ability of *E. coli* O157 to survive throughout the 21-day period, with the strain from the seventh

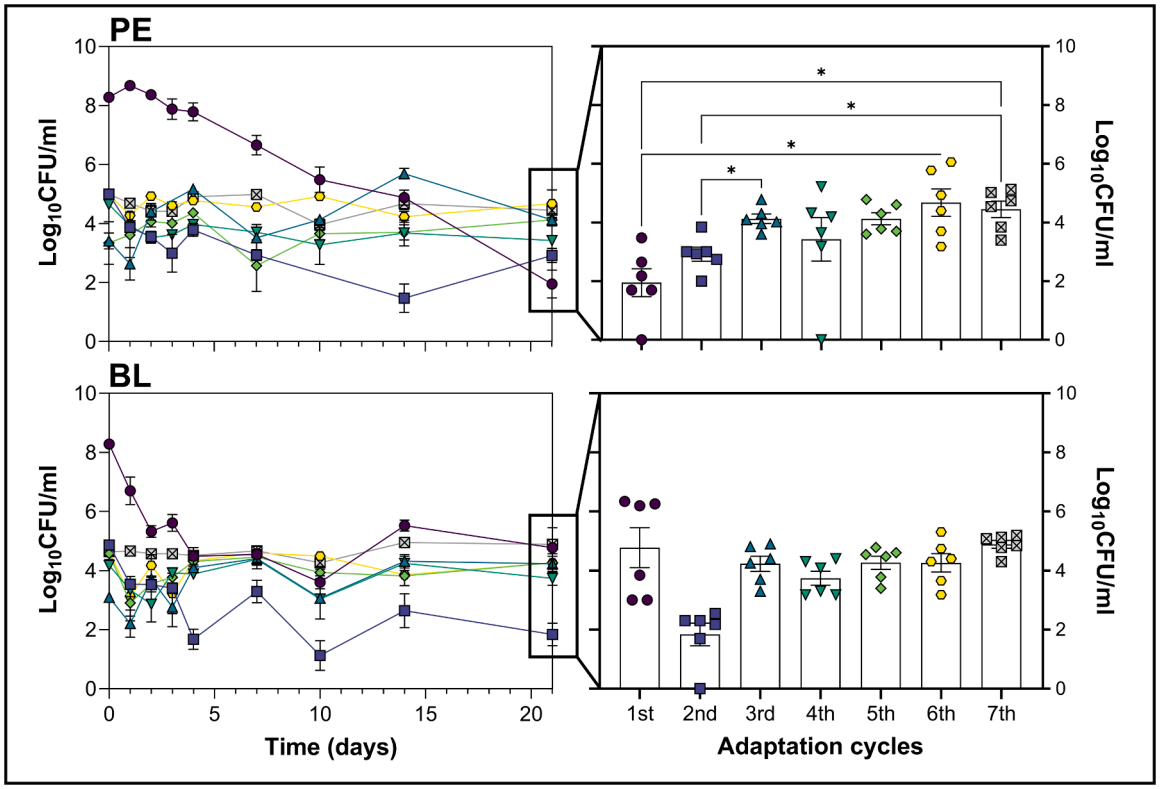

Fig. 3. Effect on *E. coli* O157 persistence following repeated exposure to polyethylene (PE) and banana leaf (BL) biofilms. Data are presented as Log_{10}CFU/ml following the recovery of *E. coli* O157 from PE and BL during a 21-day period. Four days into each adaptation cycle, *E. coli* O157 was removed, suspended in river water for 48 h, and used as the inoculum for the next adaptation cycle. Datapoints are presented as the mean (± SE) of six independent biological replicates. Data from the 21-day timepoint of each adapted variant of *E. coli* O157 are presented as the mean +SE. * $P < 0.05$ (one-way ANOVA with Tukey multiple comparisons post-hoc test).

cycle surviving in significantly ($P < 0.05$) greater numbers at the 21-day timepoint compared to the strains recovered after the first and second cycles. Similarly, at day 21, the strain from the sixth cycle survived in significantly ($P < 0.05$) higher concentrations than the strain from the first cycle; and the strain from the third cycle survived in significantly ($P < 0.05$) higher concentrations than the strain from the second cycle (Fig. 3). However, there were no significant differences between the strain from the third cycle and any subsequent cycles. This adaptation was not apparent for the *E. coli* O157 strains associated with the biofilms on the banana leaf, with no significant differences at the 21-day time point for any of the cycles.

3.4. Phenotypic adaptation

3.4.1. Biofilm

The concentration of biofilm formed by *E.* coli O157 following five successive plastisphere colonisation cycles was significantly greater than biofilms formed by *E. coli* O157 exposed to the plastisphere three or four times, although the concentration of biofilm did not significantly increase beyond the fifth colonisation cycle (Fig. 4a). In contrast, *E. coli* O157 recovered from the surface of the banana leaf did not show any enhanced biofilm phenotype, with no significant difference between any of the exposure time points (Fig. 4a).

3.4.2. Minimum inhibitory concentration (MIC)

The MIC against seven antibiotics was determined for wild type *E. coli* O157, and strains from the seventh colonisation cycle recovered from both PE and BL at the 21-day time point (Fig. 4b). For *E. coli* O157 recovered from PE, no significant changes in antibiotic tolerance were observed for tetracycline or erythromycin. However, there was an increase in resistance to chloramphenicol, gentamicin, tobramycin, ampicillin, and streptomycin relative to the wild-type strain. In contrast, *E. coli* O157 recovered from BL did not show increased tolerance to tetracycline or streptomycin but exhibited a slight increase in resistance to chloramphenicol, gentamicin, tobramycin, ampicillin, and erythromycin when compared to the wild-type strain.

4. Discussion

Pathogenic *E. coli* O157 can rapidly adhere to plastics and vegetation under realistic environmental conditions, particularly where there is an existing biofilm to associate with; however, here we have demonstrated that *E. coli* O157 can colonise plastic waste much more rapidly than previously considered. Importantly, environmental plastic waste presents opportunities for both colonisation and recolonisation by *E. coli* O157, which is then capable of rapidly adapting to the plastisphere by expressing an altered phenotype that provides greater ability to form biofilm and can show greater antibiotic tolerance. Taken together, these data highlight the potential for plastic waste to act as an important environmental reservoir for human clinical pathogens, and actively promote the emergence of phenotypes with greater virulence potential. This has significant implications for human health, environmental management, and mitigation strategies of plastic waste.

Rapid colonisation of plastic surfaces has been demonstrated in both clinical and food production settings, including for several important pathogenic bacterial species, for example, *S.* Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus* can adhere to microplastics within 24 h [33]. In many clinical and food settings, single-species biofilms are common; however, in environmental settings, biofilms are diverse and will continuously evolve as conditions change and species become more dominant. Our results show that in simulated environmental settings, pathogenic *E. coli* O157 can colonise plastic waste within 30 min, forming strong enough associations to withstand repeated washings. During the passage of (micro)plastics through wastewater treatment works, the high concentrations of pathogens provide significant opportunities for pathogen colonisation of plastic surfaces prior to discharge into surface waters [9]. However, in the environment, pathogens may only encounter plastics briefly, and such a transitory opportunity for interaction could reduce the chances of pathogens colonising plastic surfaces.

When plastics enter the environment, particularly water bodies, they are rapidly colonised by primary colonisers, e.g., diatoms, cyanobacteria, green algae and bacterial members of the Gammaproteobacteria (such as *Pseudomonas* spp. and *Vibrio* spp.), and Alphaproteobacteria (such as *Rhodobacter* spp.) [34], before subsequent stages of microbial succession produce a diverse biofilm community [3]. Initially, bacteria adhere to surfaces through rapid, but relatively weak interactions, which include Van der Waal forces, and hydrophobic and electrostatic interactions. Virgin plastic surfaces can be electrically charged and are often hydrophobic, which promotes the attachment of bacteria (which have hydrophobic cell walls) more readily to these surfaces. Additionally, factors including the surface texture (how weathered the plastic is), the chemical composition of the plastic, the nutrient availability, water temperature, salinity, and flow rate can all influence the initial attachment and distribution of bacteria on the plastic surface [35]. Following initial attachment, more complex mechanisms including adhesion

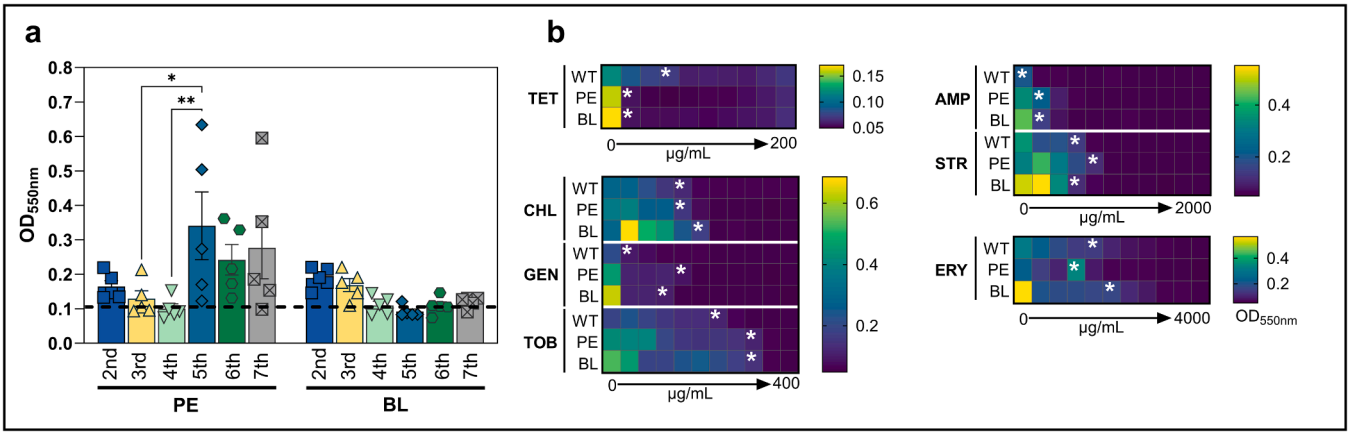

Fig. 4. Phenotypic adaptations of *E. coli* O157 following repeated plastisphere colonisation. (**a**) Biofilm concentration on polyethylene (PE) and banana leaf (BL); the dashed line in represents biofilm of wildtype *E. coli* O157 not previously exposed to PE or BL. Symbols represent five independent biological replicates and bars represent the mean (± SE). * $P < 0.05$; * * $P < 0.01$ (two-way ANOVA with Holm-Šídák multiple comparisons post-hoc test). (**b**) MIC was determined for wild type (WT) and strains of *E. coli* O157 from the seventh colonisation cycle recovered from both PE and BL at the 21-day time point. White stars represent the calculated Minimum inhibitory concentration (MIC) against tetracycline (TET); chloramphenicol (CHL); gentamicin (GEN); tobramycin (TOB); ampicillin (AMP); streptomycin (STR); and erythromycin (ERY). Colour scales are indicative of growth based on $OD_{550\ nm}$.

mechanisms such as pili, fimbriae, and flagella, firmly anchor the bacteria to the plastic surface. Bacteria then start producing EPS, which contains polysaccharides, proteins, lipids, and DNA, forming a protective and adhesive matrix that further stabilises the attachment [36]. The colonisation rate of *E. coli* O157 on both the PE and BL was further increased by the presence of organic material in the original inoculum, which would also be ubiquitous in sewage and aquatic environments. Organic material in the inoculum likely enhanced colonisation by adsorbing bacterial cells onto its surface before adhering to the PE and BL; bacterial cells can also become encapsulated with a gel-like matrix of organic polymers [37].

In aquatic environments, suspended organic matter rapidly attaches to substrate surfaces, and this process may prime plastic surface for subsequent microbial colonisation [38]. This conditioning film, or 'eco-corona' comprises proteins, lipids, and other biomolecules from the surrounding biota, and significantly modifies the physicochemical properties of the plastic surface, transitioning it from hydrophobic to more hydrophilic, and thus enhancing the adsorption capacity for microorganisms and environmental contaminants. Therefore, it has been suggested that plastics do not induce plastisphere formation but instead the plastisphere is an association with the eco-corona binding to the surfaces of plastics [39,40].

In this study, the presence of a plastisphere biofilm enhanced the ability of pathogenic *E. coli* O157 to associate with the plastic surface, relative to virgin materials. This is likely due to the abundant EPS biofilm matrix that creates a highly adhesive surface, which can attract and retain bacterial cells more effectively than surfaces without a biofilm. Biofilms are inherently hydrophobic and therefore stimulate hydrophobic-hydrophobic interactions with planktonic bacteria [41], which leads to the rapid colonisation of environmental plastic pollution and the potential for subsequent colonisation by pathogens. Once associated with the biofilm, bacteria gain some degree of protection from fluctuating environmental stressors such as temperature, UV irradiance, and desiccation. Subsequent changes in genetic and phenotypic profiles of bacteria in the biofilm can increase subsequent colonisation, enhance opportunities for cell-cell communication and the coordinated release and detection of molecules capable of further increasing adherence and biofilm cohesive strength [42,43].

Bacterial adaptation is a critical process, that allows bacteria to survive and persist under a range of different stressors [44]. Adaptation involves rapid and dynamic changes in response to the physical, biological, and chemical properties of the surfaces that they encounter, and is achieved through mechanisms involved in physical attachment, gene regulation, surface sensing, stress responses, phenotypic changes, and HGT [45,46]. Many pathogenic bacterial species employ adaptative strategies to gain competitive advantages both for survival and infectivity. For example, in biofilms, *E. coli* can increase production of curli fibres and cellulose to increase surface colonisation and form denser biofilms [47]; and small-colony variants (SCVs) of *S. aureus* that are produced during biofilm growth are more resistant to antibiotics and immune system attacks [48]. In this study, we have shown that repeated plastisphere exposure gives *E. coli* O157 the ability to dissociate from, but then has an increased capacity to recolonise the plastisphere over subsequent generations, which may allow a competitive advantage within the plastisphere. This trait was also evidenced through in vitro biofilm formation assays, where *E. coli* O157 recovered from the plastisphere showed an enhanced ability to form biofilm (relative to wild type), suggesting that repeated exposure to the plastisphere could be selecting for superior biofilm-forming variants. Biofilm often contains dense pockets of concentrated nutrients and areas of nutrient limitation, which can lead to the selection of variants that are more efficient at utilising limited resources and persisting in a dormant state [49,50]. Repeated exposure to the plastisphere could also select for spontaneous mutants with an enhanced ability to form biofilm, for example, repeated biofilm formation by *P. aeruginosa* can result in mutations in *gacS/gacA*, which increases the production of biofilm matrix components [51]. The specific effect of exposure to plastisphere biofilms on the genetic manipulation of pathogenic bacteria is unclear but would allow a greater understanding of the role of plastic pollution in the potential emergence of novel variants.

Importantly, *E. coli* O157 showed enhanced antibiotic resistance following repeated exposure to both PE and BL biofilms. While biofilms can act as barriers to antibiotic penetration, they can also sequester antimicrobial compounds within the biofilm matrix [52]. This can lead to an antibiotic concentration gradient that decreases with the depth of the biofilm [53]. This means that pathogens can be frequently exposed to sublethal concentrations of antimicrobials within the biofilm, which can promote the evolution of more tolerant variants better able to withstand these compounds [54,55]. In most cases, this tolerance and resistance is developed through genetic mutation, e.g., mutations in regulatory genes like *rpoS* (stationary phase sigma factor) that can enhance stress responses and biofilm formation [56]. However, bacteria can acquire genes from other bacteria through HGT via transformation, transduction, conjugation, and mobile genetic elements (MGEs) [57]. Following exposure to antimicrobials, the plastisphere shows an increased tolerance to antibiotics, and selects for antibiotic resistance bacteria that encode antimicrobial resistance genes, virulence factors, and genes involved in plasmid conjugation [58].

5. Conclusions

Pathogenic bacteria can rapidly colonise, adapt to, and evolve on the surface of plastic waste, increasing their environmental persistence and posing a potential threat to public health. The growing abundance of plastic waste in the environment enhances the likelihood of pathogen-plastic interactions, thereby heightening the co-pollutant risk to humans. Moreover, the long-lasting presence of plastic waste in the environment provides repeated opportunities for colonisation and recolonisation by both pathogenic and commensal species, further facilitating the exchange of genetic information and the evolution of more virulent variants. After interacting with the plastisphere, phenotypic adaptation can result in enhanced virulence-associated traits, underscoring the potential for environmental plastic waste to significantly impact human health. This study emphasises the urgent need for improved mitigation and intervention strategies to address plastic pollution, together with continued research into the risks associated with the selection of pathogenicity traits and pathogen evolution in response to environmental pollutants.

Environmental implications

The abundance of plastic in the environment offers significant opportunities for pathogenic bacteria to colonise the surfaces of plastic pollution, which can increase the potential for their dissemination and subsequent contact with humans. This study demonstrates that pathogenic bacteria can rapidly (within 30 min) colonise plastic waste following short exposure times, and that repeated colonisation of plastic waste encourages the emergence of variants with enhanced virulence traits. With the increasing emergence of novel and more virulent pathogens of serious global concern (particularly those encoding antimicrobial resistance), understanding the co-pollutant risk of pathogens and plastics, and their combined potential to impact human health, is essential for developing appropriate public and environmental management interventions.

CRediT authorship contribution statement

Rosie Fellows: Writing – review & editing, Data curation. **Richard Quilliam:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hannah White:** Writing – review & editing, Data curation. **Luke Woodford:** Writing – review & editing, Data curation. **Michael Ormsby:** Writing – review & editing, Writing – original draft,

Formal analysis, Data curation, 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136359.

Data Availability

Data will be made available on request.

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