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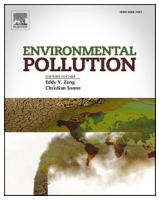

# Plastic debris facilitates the survival of multidrug-resistant bacterial pathogens in an urban agricultural environment☆

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A B S T R A C T

Rapid urbanisation in Low- and Middle-Income Countries (LMICs) has driven the expansion of urban and peri-urban farming to enhance food security. However, these systems are highly vulnerable to contaminated irrigation waters, urban runoff, open defecation and inadequate sanitation, and anthropogenic pollution, such as plastic and microplastic waste. Here, we investigated the role of plastic debris as a reservoir and vector for multidrug-resistant (MDR) enteric bacterial pathogens in a real-world agronomic setting. Focusing on two peri-urban agricultural sites in Dar es Salaam, Tanzania, we analysed 140 environmental samples (soil, water, vegetation, and surface and buried plastic debris) for the presence of four key enteric pathogens: *E. coli*, *Salmonella* spp., *V. cholerae*, and *K. pneumoniae*. The concentration of total culturable pathogens was higher on plastic debris compared to soil, water and vegetation, with presumptive *E. coli* loads of $\sim 1 \times 10^3$ CFU per individual piece of plastic debris. Importantly, plastic debris harboured a greater proportion of MDR strains; specifically, 69% of *E. coli* isolates were resistant to two or more antimicrobials, with plastics at one site accounting for over half of all MDR *E. coli*. While MDR *E. coli* were absent from soil, plastic debris supported strains of *E. coli* and *K. pneumoniae* that were resistant to critically important antimicrobials (e.g., ciprofloxacin and cefixime).This study provides robust evidence that in a real-world setting, plastic waste can act as an ecological reservoir which concentrates and facilitates the survival of MDR pathogens. Therefore, the widespread presence of contaminated plastic in agricultural systems could pose significant occupational health risks for farmers, in addition to a potential environment-to-food risk for consumers.

## 1. Introduction

Worldwide, more than 800 million farmers are engaged in urban agriculture, and of these, about 200 million practice market-oriented farming (Qadir et al., 2010). In many low-and-middle-income countries (LMICs), urban and peri-urban farming is rapidly expanding, driven by increased urbanisation, food security concerns, and economic opportunities (Pawlak and Kołodziejczak, 2020). Urban and peri-urban farmers in LMICs can enhance household income by producing perishable crops such as leafy vegetables for sale in local markets, which are crucial for providing a continual supply of vitamin-rich vegetables to the community (Silva Dias, 2011). Farmers often prefer to use river water for irrigation as it provides a free source of nitrogen and phosphorus (and thus, less money spent on fertilisers) and can be more reliable or cheaper than other water sources (Weerahewa et al., 2023; Olkeba et al., 2025). However, rivers and streams flowing through urban areas, are frequently contaminated by inorganic pollutants such as heavy metals, and organic pollutants such as pharmaceuticals and (micro) plastics, together with significant inputs of pathogenic organisms (Qadir et al., 2010; Kurwadkar, 2019; Osman et al., 2024). As a result, agricultural soils in LMICs are frequently linked to disease outbreaks due to the use of polluted irrigation water (Ali et al., 2023; Niquice-Janeiro et al., 2024).

Globally, agricultural soils are becoming increasingly contaminated with plastics, both from agricultural practices, e.g., from degraded plastic mulches, and their introduction via flooding and runoff from adjacent urban settlements (Pow et al., 2025). While the impacts of plastics on crop yields, nutrient uptake, and overall plant health remain contentious, especially at environmentally relevant concentrations, it is

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the emerging role of plastics in disease transmission in these settings that presents an as yet, unquantified risk (Zhu et al., 2022; Thomas et al., 2024; Woodford et al., 2024a). The surfaces of environmental plastics are increasingly recognised as a significant habitat for diverse microbial communities (Zettler et al., 2013), which often includes pathogenic bacteria, fungi, and viruses (Gkoutselis et al., 2021; Metcalf et al., 2022; Moresco et al., 2022). This so-called 'plastisphere' can also selectively enrich pathogens from the surrounding environment (Pham et al., 2021; Junaid et al., 2022) and provides a protective niche that can shield microbial communities from harsh environmental factors and thus enhance their survival and dissemination (Amaral-Zettler et al., 2020). Real-world studies in LMICs have confirmed the ubiquitous presence of major enteric bacterial pathogens on plastic waste (Mphasa et al., 2025), and as frequent contaminants of drains, standing water, and soil in agricultural settings (Mwapasa et al., 2024). Moreover, soil and crop mesocosm studies using simulated environmental conditions have demonstrated the protective role of the plastisphere for enteric pathogens such as *Salmonella* Typhimurium and *Vibrio cholerae* in agricultural settings (Woodford et al., 2024a; Woodford et al., 2024b).

Infectious diseases pose a substantial global health burden, with bacterial pathogens accounting for a large proportion of morbidity and mortality. Globally, sub-Saharan Africa faces the highest mortality rate from bacterial disease, with 230 deaths per 100,000 population (Ikuta et al., 2022). Enteric pathogens, typically transmitted via the faecal-oral route, contribute significantly to these infections, often facilitated by inadequate sanitation infrastructure. Given the emerging evidence that plastic waste in urban informal settlements of LMICs have the potential to serve as environmental reservoirs for pathogenic bacteria (Mphasa et al., 2025), there is now an urgent need to quantify the role of plastics as vectors for pathogens in urban agricultural settings.

Human exposure to pathogens in the plastisphere could occur through handling contaminated soil and crops, from exposure to contaminated microplastics adhering to produce, or from pathogen transfer from the surfaces of plastics to the surfaces of crops (Quilliam et al., 2023; Woodford et al., 2024b). Here, we have carried out a real-world field-scale sampling campaign to quantify the presence of enteric bacterial pathogens on plastics in irrigation-fed agricultural areas in Dar es Salaam, Tanzania. Specifically, our aim was to: (1) quantify the presence of enteric bacteria on plastic debris, soil, vegetation, and in river water used for irrigation at peri-urban agricultural sites; and (2) quantify the level of antimicrobial (AMR) and multidrug (MDR) resistance of enteric bacteria associated with the plastisphere in these agricultural settings.

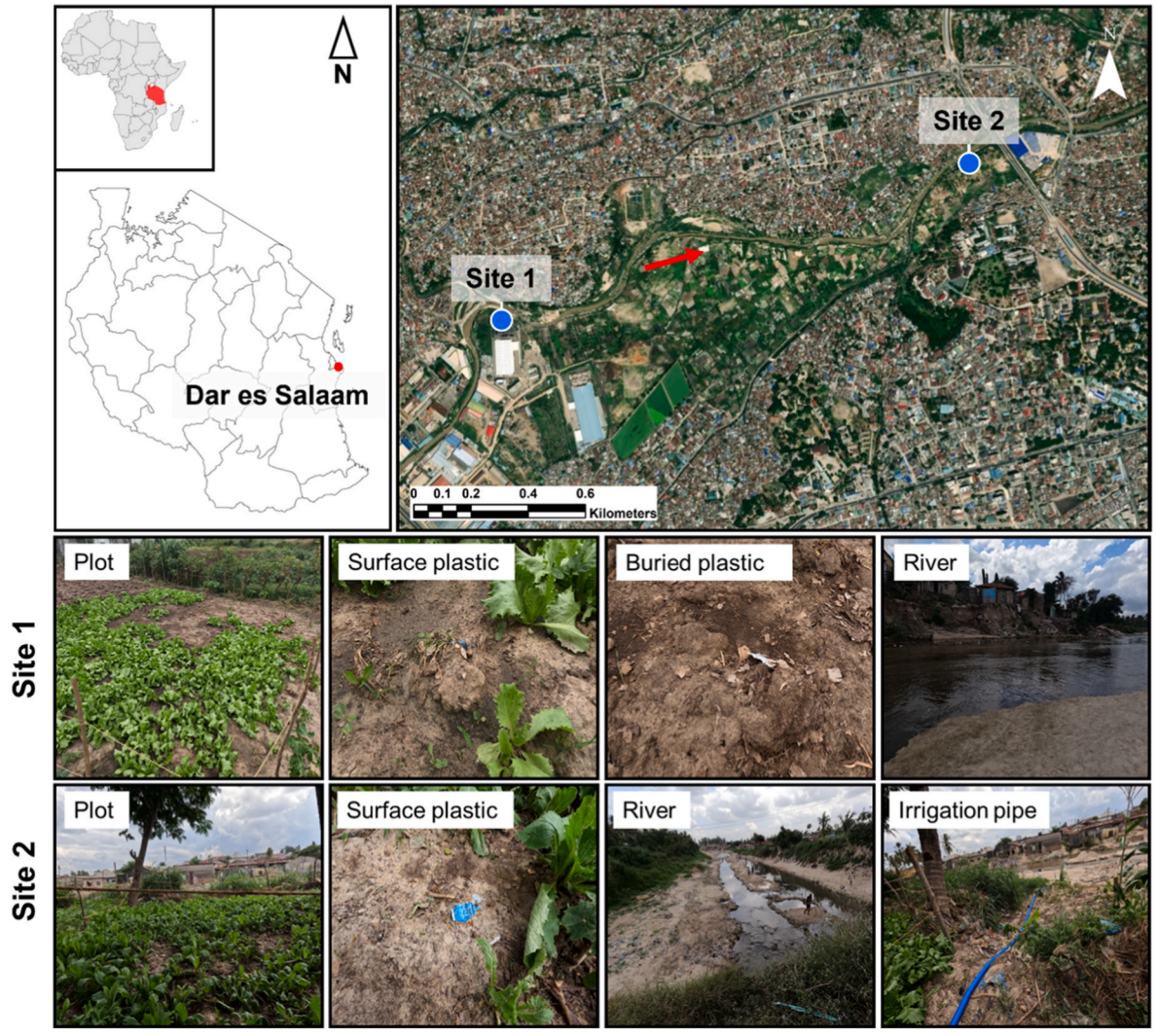


**Fig. 1.** Agricultural sampling sites in Dar es Salaam, Tanzania. Surface plastics, buried plastics, soil, vegetation, and river water were collected from each site in July 2024. Site 1 is in the Sukita district, with lettuce grown at the time of sampling, and Site 2 is in Amana, next to the Kigogo township, with Chinese cabbage (*Brassica rapa*) grown at the time of sampling. The red arrow indicates the flow direction of the river. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## 2. Materials and methods

### 2.1. Location of sampling sites and sampling strategy

Two agricultural sites along the Msimbazi river, Dar es Salaam were sampled in July 2024. Site 1 (−6.824119, 39.240257; 4.9 $m^2$ of cultivated area) is in the Sukita district, with lettuce grown at the time of sampling, and Site 2 (−6.819721, 39.254233; 6.1 $m^2$ of cultivated area) is in Amana, next to the Kigogo township, with Chinese cabbage (*Brassica rapa*) grown at the time of sampling (Fig. 1). Both sites are adjacent (<30 m) to the Msimbazi River, which is the primary source of water used for irrigation in these areas. Sampling locations within each site were chosen using a convenience sampling approach, targeting areas with visible plastic accumulation (defined here as macro-debris >0.5 cm in size) and active river irrigation use (defined as the regular, manual or mechanical application of river water for crop maintenance). At each site, surface plastic debris ($n = 35$) was collected non-selectively from the top 1 cm of soil using sterile forceps and immediately placed into sterile sampling bags to prevent cross-contamination. In this study, an individual "piece" of plastic was treated as the primary functional unit of contamination, reflecting how plastic debris is encountered and managed in real-world agricultural settings. Control samples of soil ($n = 10$), river water ($n = 10$), and local vegetation ($n = 10$) were also collected at each site. The higher sample size for plastic debris was prioritised to capture the significant spatial and morphological heterogeneity inherent to the plastisphere, while the other environmental matrices provided a representative baseline of background contamination levels. Recovered fragments were rinsed with sterile deionised water to remove loosely adhered soil and subsequently categorised into polymer classes based on standard macroscopic physical properties. This classification process followed a systematic criteria: Low-Density Polyethylene (LDPE) was identified by its characteristic thin, flexible film structure (e.g., shopping bags/irrigation sheeting); Polypropylene (PP) by its presence as woven fibres or twine (e.g., produce sacks); and High-Density Polyethylene (HDPE) by its rigid, opaque structure and higher tensile strength. Where available, manufacturer-embossed recycling codes were used to confirm these physical assignments. As an exploratory measure, recovered plastic debris was also categorised by primary colour to investigate potential links between pigmentation (as a proxy for specific chemical additives or differing rates of photo-degradation) and microbial colonisation. To collect buried plastics, a 25 cm deep trench was excavated, and visible plastic fragments between 10 and 20 cm depth were carefully removed using sterile forceps. The soil at both sites was characterised as sandy-loam. Additionally, surface soil ($n = 5$), and soil from a depth of 10-20 cm ($n = 5$), leaves of the crop ($n = 5$) and river water ($n = 5$) were collected to provide a minimum representation of the local environment at the time of sampling. Temperature, pH, and electrical conductivity (EC) of the soil, and the pH and EC of the river water were recorded *in situ* at each site (Table S1).

### 2.2. Sample collection and processing

All samples were collected into sterile tubes and kept on ice in insulated coolers (~4 °C) during transport to the lab and were processed within 24 h of collection. To remove loosely attached soil and microorganisms each individual piece of plastic and vegetation was suspended in 5 ml sterile $dH_2O$, inverted three times, and the water discarded. Five sterile glass beads (approx. 5 mm in size) and 2 ml of PBS was added to every replicate tube of soil, vegetation, and plastic pieces (only glass beads were added to the tube containing the river water), and all tubes vortexed for 30 s to promote disruption of the biofilm. For vegetation samples, it is acknowledged that this mechanical disruption likely released both surface-associated (epiphytic) and a portion of internalised (endophytic) bacteria; therefore, the resulting CFU counts represent a composite plant-associated microbial signal. Finally, 50 μl of the suspension from each tube was spread onto the surfaces of three separate Petri dishes containing either MacConkey agar (Oxoid, UK), thiosulfate–citrate–bile salts–sucrose agar (TCBS; ThermoFisher, UK), or *Salmonella/Shigella* agar (SS; ThermoFisher, UK), to allow selective and quantitative isolation of presumptive *E. coli*, *V. cholerae* and *Salmonella* spp., respectively. Plates were inverted and incubated at 37 °C for 24 h followed by the enumeration of colony forming units (CFU) of presumptive *E. coli* (red/pink colonies on MacConkey agar), *V. cholerae* (flat, 2-3 mm in diameter, yellow colonies on TCBS), and *Salmonella* spp. (colourless colonies with black centres on SS agar). From each plate, two single colonies were picked and enriched in LB broth, at 37 °C. After 24 h, 400 μl of culture was removed and added to 80% glycerol in LB and stored at −80 °C for subsequent AMR analysis.

### 2.3. Species confirmation through VITEK mass spectrometry and targeted PCR

To confirm the culture-based identification of each presumptive *E. coli* isolate, isolates were first subjected to VITEK-MS (bioMérieux, France) for species identification, followed by further molecular confirmation. This targeted approach was taken due to well described inconsistencies in VITEK-MS identifications for certain bacterial groups. For instance, many isolates presumptively identified as *E. coli* by culture were identified as *Klebsiella pneumoniae* by VITEK-MS, highlighting the common challenge of distinguishing these closely related bacterial species based solely on MacConkey growth characteristics. Consequently, molecular confirmation via PCR was conducted for all *E. coli* and *K. pneumoniae* isolates.

Given the established specificity of the *uidA* gene for *E. coli*, all isolates presumptively identified as *E. coli* by VITEK-MS were subsequently confirmed via PCR amplification targeting the *uidA* gene. Additionally, a PCR targeting the bacterial 23S ribosomal RNA gene (*rrl*), a highly conserved gene present across diverse bacterial species, was performed on all isolates as a general bacterial confirmation. Based on these results, isolates were categorised as follows: those presumptively identified as *E. coli* by VITEK-MS and positive for both *uidA* and *rrl* were confirmed as *E. coli*. Isolates presumptively identified as *Klebsiella pneumoniae* by VITEK-MS that were negative for *uidA* but positive for *rrl* were confirmed as *K. pneumoniae*. PCR reactions were carried out in a thermocycler (Applied Biosystems, USA) using published primers (Table S2). PCR runs included a no-template control and positive control DNA for each primer pair.

Isolates recovered on TCBS and SS agar were not subjected to VITEK-MS or further molecular confirmation and are therefore reported here as presumptive *Vibrio* spp. and presumptive *Salmonella* spp., respectively. This targeted confirmation strategy allowed for the prioritisation of high-resolution molecular and proteomic analysis on the *E. coli* and *K. pneumoniae* isolates, which formed the basis of the AMR profiling.

### 2.4. Antimicrobial resistance and susceptibility testing

Antimicrobial resistance and susceptibility of confirmed *E. coli* and *K. pneumoniae* isolates were quantified using the Kirby-Bauer disc diffusion assay. Briefly, glycerol cultures of each isolate were plated onto nutrient agar and grown overnight at 37 °C. Pure colonies were selected and resuspended in 5 ml sterile saline solution to obtain a turbidity of 0.5 McFarland standard. Cells were then plated on Muller-Hinton agar (Oxoid, UK). Discs (all Oxoid, UK) were applied to the surface of the agar followed by incubation at 37 °C for 18 h. A total of five antibiotic discs (Oxoid) were applied: amoxicillin–clavulanate (AMC, 30 μg), cefixime (CFM, 5 μg), ciprofloxacin (CIP, 5 μg), co-trimoxazole (sulfamethoxazole/trimethoprim, SXT, 25 μg), and doxycycline (DO, 30 μg). Zone diameters were interpreted using CLSI 2020 breakpoints for Enterobacterales where applicable. Specifically, where species-specific breakpoints were unavailable, CLSI recommendations for the wider Enterobacterales family were applied as the most biologically relevant proxy to ensure a consistent standard for determining

resistance within this taxonomic group.

All disk diffusion assays included positive control strains of *E. coli* and *K. pneumoniae* as controls. We define MDR as resistance to three or more antimicrobial classes, consistent with widely used definitions (Magiorakos et al., 2012).

### 2.5. Statistical analysis

Differences in CFU concentrations between substrates (plastics vs. soil vs. water vs. vegetation) and between sites (S1 vs. S2) were analysed using a two-way Analysis of Variance (ANOVA), followed by a post-hoc Kruskal-Wallis test for multiple comparisons. Statistical analyses were performed using GraphPad Prism (Version 10), and a significance level of $P < 0.05$ was used for all tests.

## 3. Results

### 3.1. Culture-based identification

Culture-based assays revealed a heterogeneous distribution of presumptive *E. coli*, presumptive *Vibrio* spp., and presumptive *Salmonella* spp., across the diverse environmental substrates sampled: plastic debris, soil, vegetation, and river water (Fig. 2). Presumptive *E. coli* emerged as the most frequently detected bacterial group, with substantial contamination (~$1 \times 10^3$ CFU per individual piece of material) commonly observed on the surface of plastic debris on agricultural soil. River water was consistently contaminated with presumptive *E. coli* (Site 1 average: $5.4x10^3$ CFU/ml; Site 2 average: $9.1x10^2$ CFU/ml), and presumptive *V. cholerae* (Site 1 average: $3.68x10^3$ CFU/ml; Site 2 average: $1.2x10^4$ CFU/ml). Furthermore, presumptive *V. cholerae* was detected in agricultural soil (10-20 cm depth) (Site 1 average: $1.6x10^3$ CFU/ml; Site 2 average: $3.9x10^3$ CFU/ml). In contrast, presumptive *Salmonella* spp. was detected less frequently and generally at low

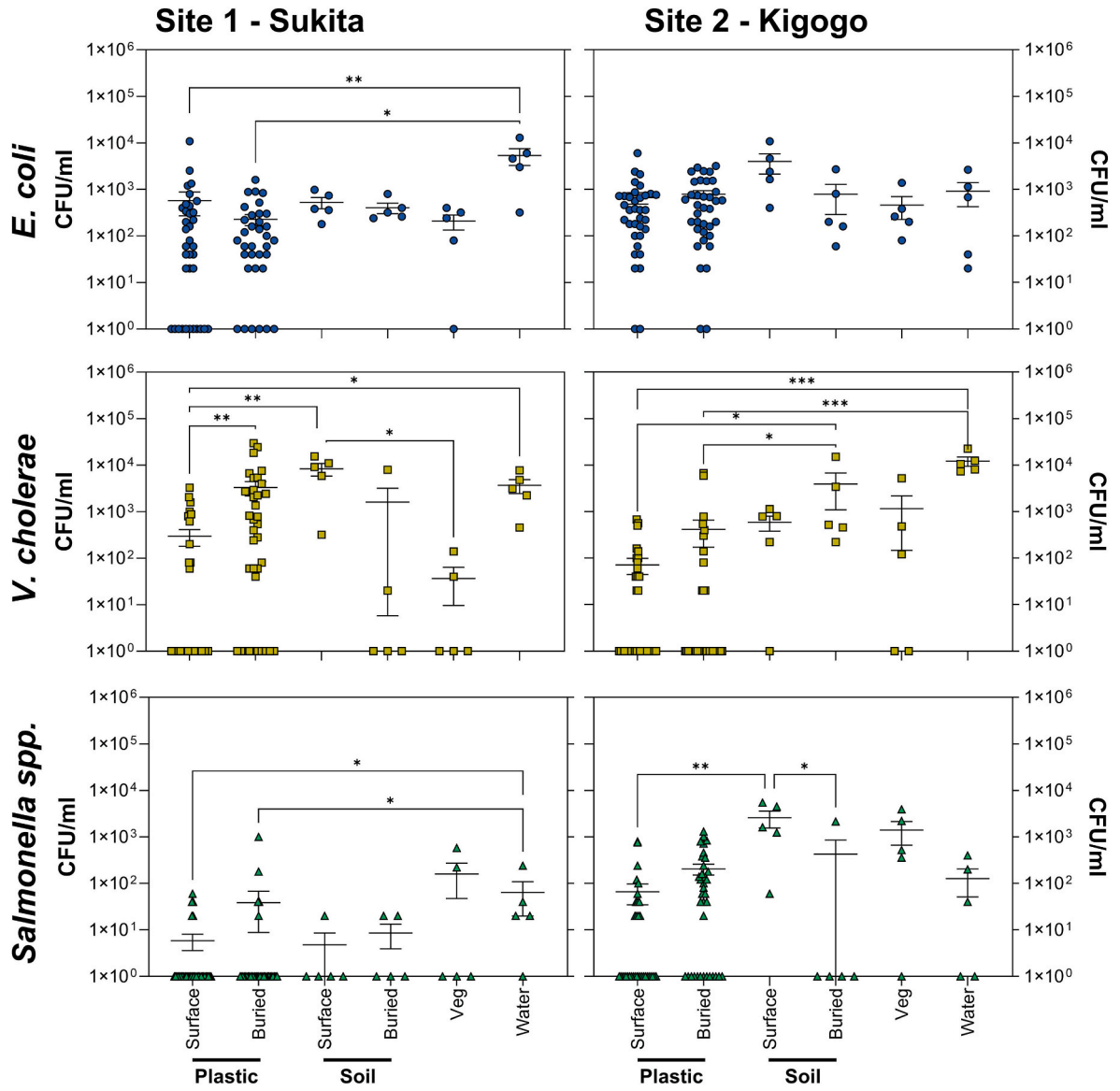


**Fig. 2.** Culture-based screening of soils, plastics, crops, and irrigation water for enteric pathogens. Data points represent individual biological replicates (i.e. individual pieces of plastic or samples of soil, leaves or water collected at each site) Error bars represent the mean ± SD. Viable cells of each presumptive species were enumerated by plate counts. Significant differences were tested by two-way ANOVA with Kruskal-Wallis multiple comparisons post-test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

concentrations, although it was present on plastic and in soil samples at both sites.

There was a discernible difference in bacterial contamination loads between the two sites. River water at Kigogo had consistently higher presumptive *E. coli* and *V. cholerae* concentrations than river water at Sukita. Intriguingly, one surface soil sample at Kigogo showed a high co-occurrence of all three enteric bacterial groups, demonstrating the potential for multiple contamination within the soil.

Based on morphological classification, debris categorised as LDPE and PP frequently served as a substrate for presumptive *E. coli*, although concentrations varied between individual plastic pieces (Fig. 3). Colonisation of materials identified as HDPE was more variable, with some pieces, particularly from Sukita (Site 1), showing no signs of viable presumptive *E. coli*. However, where colonisation did occur on HDPE, the bacterial loads were comparable to those found on LDPE and PP, suggesting that once an initial biofilm is established, HDPE serves as an equally effective reservoir for enteric pathogens. While these categories represent the dominant physical forms of plastic observed, the lack of spectroscopic verification necessitates a cautious interpretation of polymer-specific colonisation patterns**.** The potential for plastics to be contaminated by enteric bacteria was also influenced by whether they were on the surface or buried within the soil, e.g., presumptive *Salmonella* spp., were more frequently isolated from buried plastics. From an exploratory perspective, we also assessed the relationship between fragment pigmentation and bacterial load. However, there was no correlation between the colour of the plastic and enteric bacteria in the plastisphere (Fig. 3).

All three target enteric bacteria were detected in soil at both the surface and at a depth of 10-20 cm (with particularly high concentrations of presumptive *V. cholerae* recovered in soil at 10 – 20 cm at Sukita (Site 1). Finally, all target pathogens were recovered from the surface of the two crop plants (lettuce and Chinese cabbage), with notable presumptive *E. coli* ($4.6 \times 10^2$ CFU per piece of leaf), *V. cholerae* (avg. $1.2 \times 10^3$ CFU) and *Salmonella* spp. ($1.4 \times 10^3$ CFU) concentrations on lettuce from Kigogo (Site 2).

### 3.2. Mass spectrometry (VITEK) and PCR based confirmation

A total of 159 environmental samples showed presumptive growth for *E. coli* based on colony morphology on MacConkey agar. VITEK initially identified 125 of these isolates as either *E. coli* (76 isolates) or *Klebsiella pneumoniae* (49 isolates). Further confirmation of all 125 isolates was performed using PCR targeting the *rrl* gene (for Enterobacteriaceae confirmation) and the *uidA* gene (for *E. coli* specificity), which led to the exclusion of 21 isolates that were negative for both genes. Of the remaining 64 isolates initially identified as *E. coli* by VITEK, 43 were confirmed as *E. coli* by PCR, while 21 were re-assigned as *Klebsiella* spp. Conversely, of the remaining isolates initially identified as *Klebsiella* spp. by VITEK (40 isolates), 31 were confirmed by PCR, and 9 were re-assigned as *E. coli*. In total, 104 isolates were analysed, and confirmed

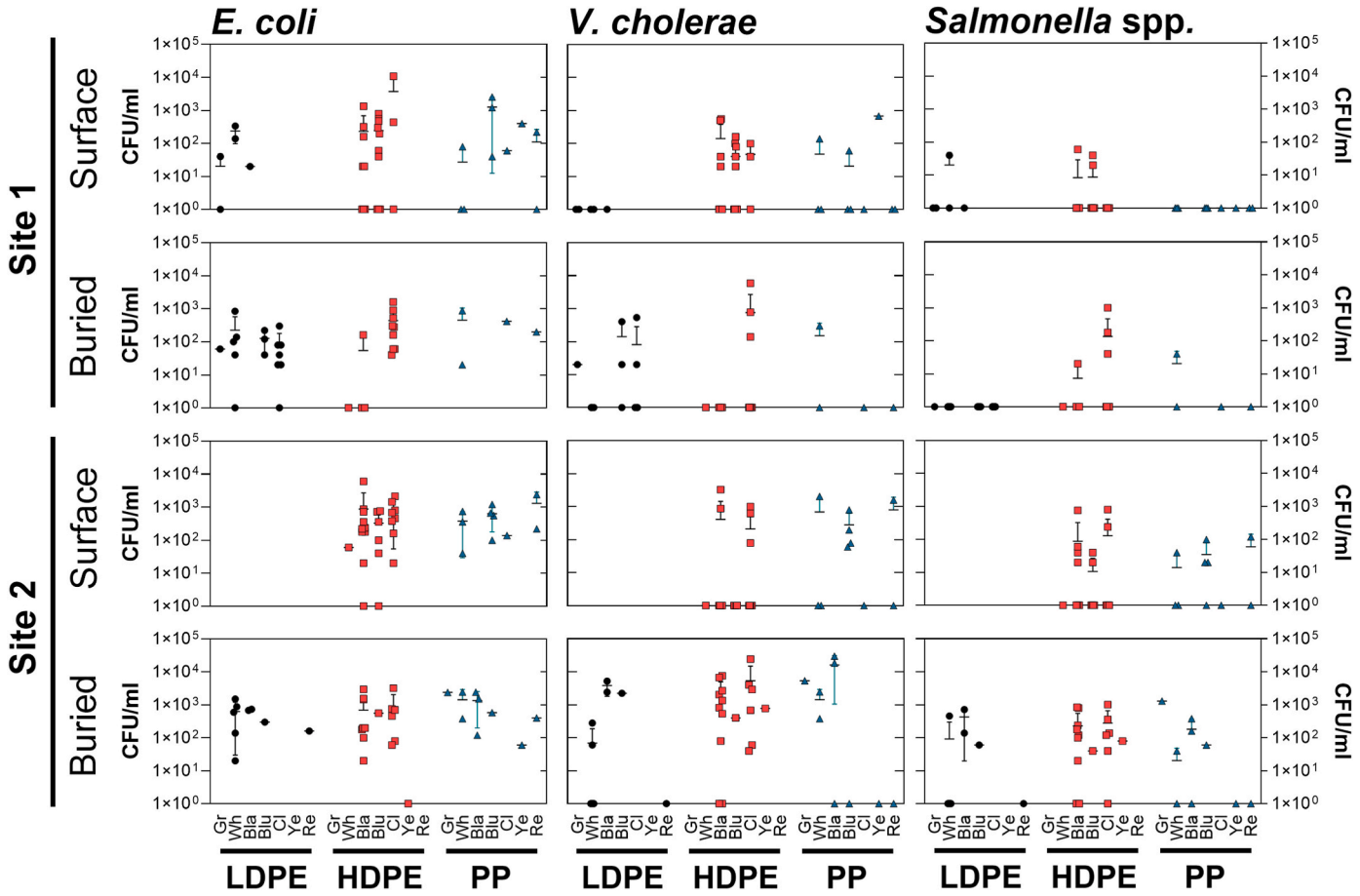


**Fig. 3.** The influence of colour and polymer type on the colonisation of enteric pathogens. Colonisation of presumptive *E. coli*, *V. cholerae* and *Salmonella* spp. on low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polypropylene (PP) collected from soil (surface and at depth) at each site. Samples were additionally sorted by prominent colour (Green [Gr]; White [Wh]; Black [Bla]; Blue [Blu]; Clear [Cl]; Yellow [Ye]; or Red [Re]). Data points represent individual biological replicates (i.e. individual pieces of plastic collected at each site). Polymer types were assigned based on physical and morphological characteristics; spectroscopic confirmation was not performed. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

as 52 *E. coli* and 52 *K. pneumoniae* isolates.

### 3.3. AMR profiles

Plastic (particularly buried plastics) were the substrates most frequently colonised by both antimicrobial-resistant strains of *E. coli* and *K. pneumoniae* but also supported the highest concentrations of susceptible strains (Fig. 4). Resistance was more common in *E. coli*, with 30 of 52 isolates (58%) exhibiting resistance to at least one of the five antimicrobials tested. Doxycycline (DO) resistance was especially prominent ($n = 30$), followed by amoxicillin (AMC; $n = 14$), with the majority of resistant isolates (18 of 30 isolates) recovered from plastic debris at Kigogo (Site 2), where buried plastics accounted for eight AMC-resistant and eight DO-resistant strains (Fig. 4). Resistance to ciprofloxacin (CIP) and cefixime (CEF) was observed less frequently ($n = 6$ and $n = 8$, respectively), while resistance to sulphonamide-trimethoprim (SXT) was scarce ($n = 3$). Isolates of *E. coli*, resistant to at least one antimicrobial, were also recovered from water ($n = 4$) and the surfaces of vegetable crops ($n = 2$).

The majority of *E. coli* isolates (92%) were susceptible to at least one antimicrobial, with the largest number of susceptible isolates recovered from buried plastics at Kigogo (Site 2), including 11 that were SXT-sensitive and 10 AMC-sensitive (Fig. 4). Intermediate susceptibility (particularly in relation to ciprofloxacin and cefixime) was identified in 22 *E. coli* isolates (42%), which were largely associated with plastic substrates, (Fig. 4). In contrast, isolates expressing intermediate phenotypes were infrequently recovered from soil, water, or crops.

Resistance in *K. pneumoniae* was less widespread, with 16 of the 52 isolates (31%) resistant to at least one antimicrobial, predominantly doxycycline ($n = 12$) and amoxicillin ($n = 6$), with those isolates associated with buried plastic surfaces accounting for the majority of resistant strains (Fig. 4). However, resistant isolates of *K. pneumoniae* were rarely recovered from water ($n = 2$) and were completely absent from soil taken at 10-20 cm depth. Fully susceptible isolates of *K. pneumoniae* were the most abundant ($n = 36$; 69%), particularly among the isolates recovered from buried plastics at Amana (Site 2), with 14 SXT-sensitive and 12 CFM-sensitive strains. Twenty *K. pneumoniae* isolates (38%) exhibited intermediate susceptibility (mainly against ciprofloxacin, doxycycline, and amoxicillin) and were largely associated with plastic surfaces (Fig. 4).

### 3.4. Multidrug resistance

Patterns of MDR further underscored the prominence of plastic surfaces as environmental hotspots for the accumulation of resistance (Fig. 5). Among *E. coli* isolates, 44 (85%) displayed resistance to at least one antimicrobial, with 36 isolates (69%) resistant to two or more, and 10 (19%) exhibiting resistance to at least four antibiotics. Plastics at Kigogo (Site 2) accounted for over half of all MDR *E. coli*. Specifically, 12 isolates from buried plastics and 11 from surface plastics at this site expressed resistance to at least one antibiotic, with six and four of these isolates, respectively, resistant to two or more antimicrobials. Three isolates recovered from the buried plastic and two isolates from the plastic debris on the soil surface were resistant to four antimicrobials, while one isolate exhibited resistance to all five. In contrast, MDR *E. coli* were absent from soil at both sites and were sparsely detected in vegetables and irrigation water ($n = 1$ and $n = 4$, respectively).

*K. pneumoniae* exhibited a similar substrate-specific pattern but with isolates exhibiting lower resistance overall. Across all sites, 23 of the 52 *K. pneumoniae* isolates (44%) were resistant to at least one antibiotic, although none exhibited resistance to more than three. As with *E. coli*, the majority of MDR strains were associated with plastics, particularly buried plastics at Kigogo (Site 2), which yielded six isolates resistant to one antimicrobial, three isolates resistant to two, and one resistant to three antimicrobials. No *K. pneumoniae* isolates exhibited resistance to four or more antibiotics, and no resistance was detected in soil at 10-20

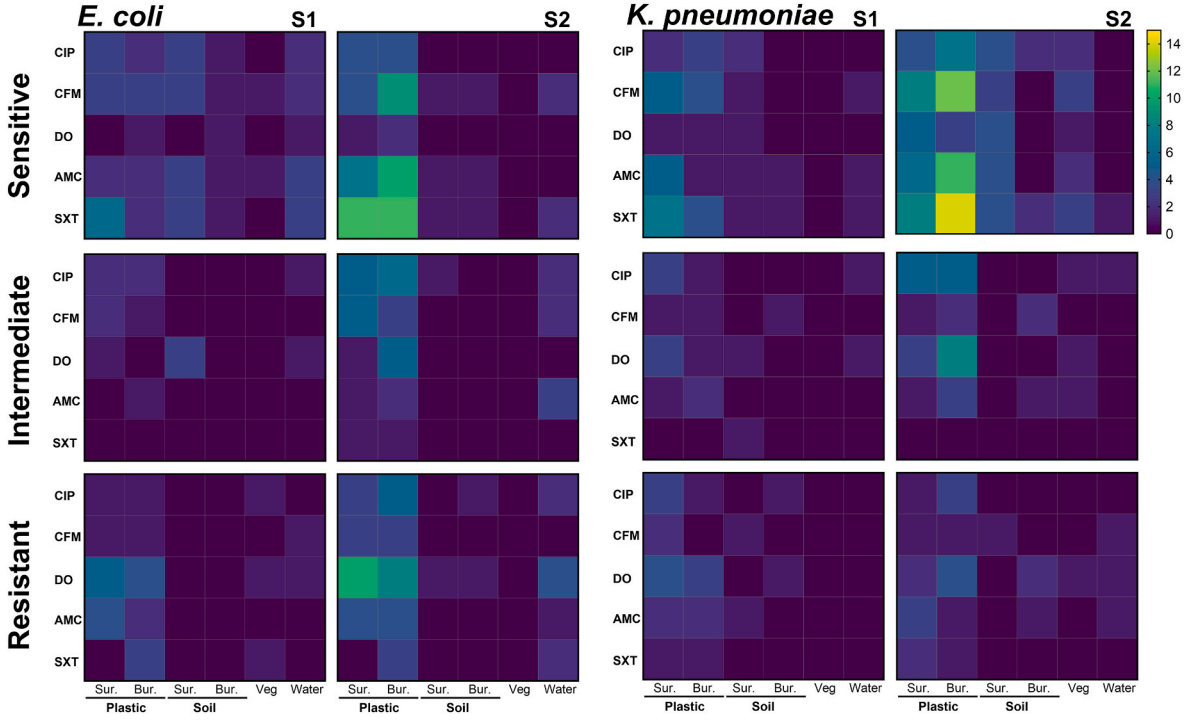


**Fig. 4.** Distribution of antimicrobial resistance in environmental isolates of *E. coli* and *K. pneumoniae*. Resistance of isolates recovered from plastics and soil at the surface (sur) and at depth (dep); and from vegetables (veg) and irrigation water at Site 1 (S1) and 2 (S2) was determined against ciprofloxacin [CIP]; cefixime [CFM]; doxycycline [DO]; amoxicillin [AMC]; and sulphonamide trimethoprim [SXT]. The colour scale indicates the number of isolates in each category. Sensitivity, resistance and intermediate resistance was determined by Kirby-Bauer disc diffusion assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

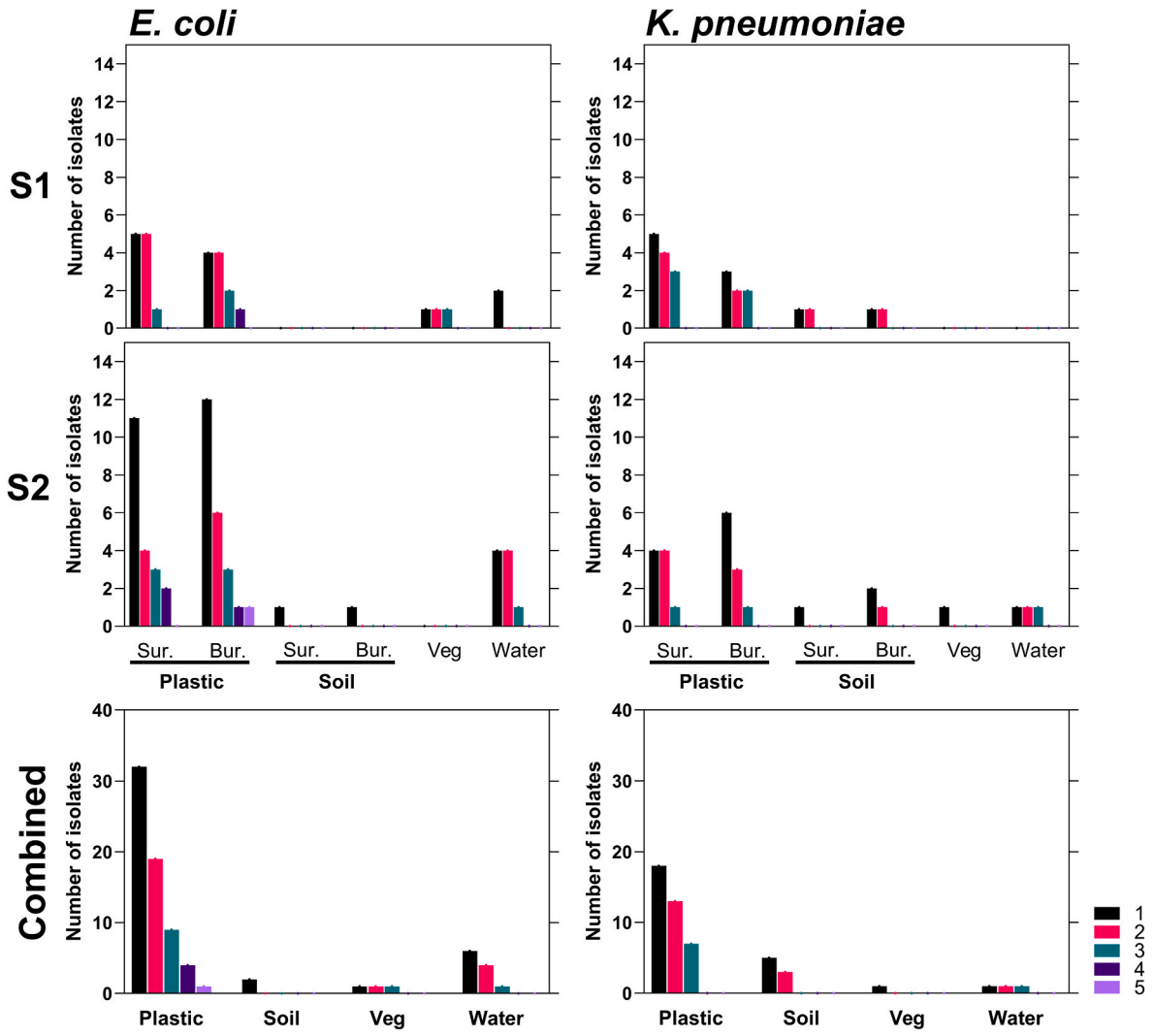


**Fig. 5.** Distribution of multidrug resistance of environmental isolates of *E. coli* and *K. pneumoniae*. Multidrug resistance of isolates recovered from four distinct environmental substrates: plastic debris (from the surface [sur] and buried [bur]), soil (surface and at depth [dep]), vegetables (veg), and irrigation water, sampled across two sites (Site 1: S1, and Site 2: S2). Bars show the total number of isolates tested for each matrix, categorised by the number of antimicrobials to which they were resistant. The five colours correspond to resistance against one (black), two (red), three (green), four (dark purple), or five (pink) antimicrobials. Related to (Fig. 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cm depth or associated with the vegetation of the crop. A small number of resistant isolates were recovered from irrigation water ($n = 3$) and surface soil ($n = 2$), but these were exclusively resistant to just one or two antibiotics.

## 4. Discussion

This field-scale real-world study provides evidence that environmental plastic debris is not merely an inert component of litter but potentially a dynamic public health risk amplifier, acting as a reservoir for faecal bacteria and phenotypic AMR. Importantly, our data shows that plastic debris in peri-urban agricultural fields can support and concentrate enteric MDR bacteria, with the potential to facilitate a direct environment-to-food transfer pathway. The ubiquity of plastic waste in agricultural systems could therefore contribute to the burden of infectious and AMR disease, a risk that is further amplified by the limited sanitation and waste management resources intrinsic to many LMICs. High concentrations of *V. cholerae* in river water are indicative of faecal pollution (Awere-Duodu et al., 2025), and the pervasiveness of non-specific faecal indicators, such as *E. coli* and *Klebsiella spp.*, suggests a continuous input of human and animal waste into the river network (Holcomb and Stewart, 2020; Meradji et al., 2025). The consistently higher pathogen loads and AMR burden at the Kigogo site compared to the upstream site at Sukita, indicates a number of localised inputs and sources, most likely linked to inadequately managed sanitation and solid waste disposal in this area of high-density informal settlements. This sustained exposure may establish stronger environmental selection pressures, facilitating the enrichment of resistant populations within the plastisphere and surrounding soils. By contrast, the lower contamination observed at Sukita may indicate either reduced inputs or greater hydrological flushing, highlighting how local land-use intensity can shape the microbial ecology of agricultural landscapes.

Importantly, our data demonstrate the potential for an environment-to-food transfer pathway for enteric bacteria, evidenced by the detection of presumptive *E. coli*, presumptive *V. cholerae* and presumptive *Salmonella* spp. on the surfaces of ready-to-eat food crops such as lettuce (where cooking to eliminate pathogens is not applied) at concentrations well within the infective dose thresholds that are capable of causing infection (Schmid-Hempel and Frank, 2007). This pathway is supported by the co-occurrence of identical bacterial taxa across irrigation water, plastics, and vegetable surfaces, as well as the overlap in MDR profiles, particularly against doxycycline and amoxicillin, found in isolates from

both the plastisphere and the crops. Pathogen transfer from contaminated irrigation water can occur directly through contact with the leaves, or indirectly by splashing contaminated soil onto the leaves (Alegbeleye et al., 2018). However, our data also demonstrates that plastic debris could provide an additional pathway for pathogens to enter agricultural systems. Plastics in irrigation water already colonised by pathogens could be introduced into cropping systems, whilst plastic debris introduced into the soil from either riverine or terrestrial settings could provide habitat for subsequent pathogen colonisation (Quilliam et al., 2023). Beyond the risk to consumers, this poses an occupational health risk to agricultural workers; and due to their recalcitrance, plastics could be facilitating a long-term agronomic reservoir of pathogens and a hotspot for AMR, particularly if plastics become vertically stratified into the soil (Woodford et al., 2024a; Woodford et al., 2024b; Pow et al., 2025).

Presumptive *V. cholerae* were also prevalent on plastic debris, which may correlate with the availability of plastic as a stable environmental niche for this pathogen. Such surfaces could reflect the important role plastic pollution plays in the ecological life cycle of this pathogen, particularly in-between rainy seasons and subsequent flooding (Ormsby et al., 2024b), though, a further longitudinal study is required to determine the specific impact of plastic on its broader ecological life cycle. Conversely, the less frequent detection of presumptive *Salmonella* spp. suggests either a different environmental survival profile or a less continuous input source; however, its ability to persist in the plastisphere on buried plastic debris (Ormsby et al., 2024c), and its confirmed presence in food crops necessitates equal concern due to its low infectious dose (Teunis et al., 2010; Gharpure et al., 2021). The simultaneous recovery of both *E. coli* and *K. pneumoniae* from plastics, soil, and water further underscores the role of this peri-urban agricultural system as a pervasive and interconnected reservoir for clinically important Enterobacteriaceae.

Studies from the last decade support the hypothesis that plastic debris can act as an important ecological niche for microbial communities, with significant potential for pathogen survival and dissemination, and a hotspot for the evolution of AMR (Zhu et al., 2022; Guruge et al., 2024; Witsø et al., 2024). Unlike more hydrophilic organic matter, the hydrophobic nature of plastic polymers facilitates the rapid and stable attachment of microbes, promoting the formation of complex, protective plastisphere biofilms (Donlan, 2002; Zettler et al., 2013). While LDPE and PP were the most frequently recovered materials in this study, HDPE fragments similarly supported MDR pathogens. This is likely due to HDPE's high surface roughness and hydrophobic surface energy, which provide attachment sites and protective micro-niches comparable to those of other polyethylene-based polymers. These biofilms shield embedded bacteria from significant environmental stressors, but can also be a sink for other pollutants, such as residual antibiotics (Michaelis and Grohmann, 2023; Shree et al., 2023). This concentrated microenvironment is hypothesised to potentially accelerate HGT, offering a mechanism that could drive high rates of AMR and MDR (Abe et al., 2020; Guruge et al., 2024). In our study, the highest proportion of resistant and intermediately susceptible strains of enteric bacteria were consistently isolated from the surfaces of plastic debris, particularly those buried within the soil. Buried plastics are likely to experience reduced UV exposure, more stable temperature and moisture conditions, and limited oxygen diffusion, factors that together create a comparatively protected microenvironment. Such conditions would favour biofilm maturation and enteric bacterial persistence due to a reduction in environmental stress-induced mortality (Ormsby et al., 2024c; Woodford et al., 2024a). Previous studies have demonstrated that the subsurface soil environment provides a buffering effect against the high-frequency environmental fluctuations observed at the surface, thereby facilitating more stable biofilm development on plastic substrates (Amaral-Zettler et al., 2020; Pow et al., 2025). The reduced disturbance of buried plastic debris may also promote sustained interactions between soil and plastisphere communities, enhancing opportunities for HGT and the maintenance of AMR determinants over extended timescales. Furthermore, the protective niche of the plastisphere could maintain pathogen viability during several cropping cycles, creating a long-term contamination source that could even lead to the selection of more virulent variants (Ormsby et al., 2024a).

The majority of *E. coli* isolates were resistant to at least one of the five antimicrobials used in this study; however, some isolates remained fully susceptible to all antibiotics tested. Resistance was most prominent against doxycycline and amoxicillin, followed by cefixime and ciprofloxacin. Doxycycline and amoxicillin are widely used, easily accessible, and often first-line antibiotics in human and veterinary medicine in LMICs (Myers et al., 2022); thus, such high resistance rates (especially in *E. coli*) probably reflect adaptation to these drugs in the environment (with high concentrations of antimicrobials likely coming from mismanaged wastewater and faecal wastes). Importantly, ciprofloxacin (a fluoroquinolone) and cefixime (a third-generation cephalosporin) are often considered important therapeutic options or second-line agents for treating severe enteric infections and septicaemia caused by bacteria, especially when first-line treatments fail or the pathogen is suspected to be resistant (Stoesser et al., 2013). The isolation of MDR strains (already resistant to these critical antimicrobials) in the plastisphere indicates that plastic debris is actively hosting pathogens that could compromise clinical treatments. We suggest that plastisphere communities may also be acting as an evolutionary bridge, e.g., by facilitating HGT with other bacteria within the plastisphere, strains with intermediate susceptibility may develop full resistance.

*K. pneumoniae* is a well-recognised cause of severe community-acquired and nosocomial infections, including pneumonia, septicaemia, and urinary tract infections (Abbas et al., 2024), and its presence in agricultural soil and irrigation water further underscores the clinical significance of these findings. Its recovery from plastic debris in agricultural fields suggests that environmental reservoirs could be acting as overlooked sources of clinically important lineages, particularly given the capacity of *K. pneumoniae* to acquire extended-spectrum β-lactamase (ESBL) genes (Silago et al., 2021). Although resistance levels were generally lower than those of *E. coli*, this may reflect differing environmental tolerances or exposure histories rather than reduced risk. The consistent recovery of *K. pneumoniae* from buried plastics emphasises the need to monitor this species alongside *E. coli* within environmental AMR surveillance frameworks.

The consistent association of AMR strains with plastics presents a considerable opportunity for wider transmission and thus an increased potential for human exposure. The lightweight and buoyant properties of plastic debris makes them highly mobile; once colonised by microbial biofilm, waterborne plastics can act as dissemination vectors, physically transporting resistant bacterial pathogens over large distances and potentially across geographical barriers, e.g., when contaminated river water is used for irrigation. Plastic polymers are slowly degraded in the environment and therefore provide a substrate for continual re-contamination over decades or even centuries (Chamas et al., 2020; Ormsby et al., 2024a). The comparatively rougher surface structure and lower crystallinity of LDPE and PP polymers that were present in the agricultural fields of our study promote bacterial attachment, while the leaching of plasticisers and additives may provide supplementary carbon sources that encourage biofilm development (Romera-Castillo et al., 2022). The absence of a clear relationship between plastic colour and bacterial load suggests that surface chemistry and texture exert a stronger influence for colonisation than colour.

Although this study provides robust evidence for the role of plastics in concentrating AMR pathogens in agricultural system, sampling was restricted to only two peri-urban sites and was carried out at only a single time point. Additionally, while polymer types were assigned based on morphological and physical traits common to local waste streams, the lack of spectroscopic confirmation (e.g., FTIR or Raman) is a pragmatic limitation of this field-scale study. Furthermore, while selective media provided an effective screen for *Salmonella* and *Vibrio*

loads, the absence of molecular confirmation for these specific genera introduces a degree of taxonomic uncertainty common in large-scale environmental surveys. We have addressed this by strictly reporting these isolates as "presumptive" to ensure that the results are interpreted with the appropriate caution regarding potential false positives from closely related environmental taxa.

While culture-dependent methods exclusively capture the viable fraction of the microbial community, thereby potentially underestimating the total pathogen burden and failing to account for the wider reservoir of ARGs (the 'resistome'), they provide a critical assessment of the pathogens posing an immediate infection risk to humans. In the context of urban agriculture in LMICs, identifying viable, MDR isolates is essential for understanding the transmission of waterborne and foodborne diseases. Despite these limitations, the consistent pattern of AMR/MDR enrichment on plastics in agronomic food-producing systems demonstrates the co-pollutant risks of plastic contaminants in agriculture.

## 5. Conclusion

This study provides evidence that environmental plastic debris acts as a key ecological reservoir and mobile vector for AMR and MDR strains in peri-urban agricultural settings. The presence of enteric bacteria, colonised onto plastic debris, presents a pathway for the direct delivery of pathogens into cropping systems, offering a mechanism for contamination that differs from well characterised waterborne routes. While these findings suggest that buried plastics represent long-term reservoirs, we acknowledge that this study represents a snapshot in time and space across two specific sites. Therefore, caution is required when extrapolating these results to broader agroecosystems, and future work should prioritise longitudinal sampling across different seasons to assess the impact of environmental variables and seasonal flooding on the dissemination of AMR from this plastic reservoir. Effective mitigation strategies must therefore address the intersection of AMR dissemination and the management of irrigation waters. Prioritising the cleanup and proper disposal of plastic debris within agricultural fields will likely reduce the potential for a persistent AMR reservoir in the plastisphere and subsequently limit pathogen dissemination. This must be coupled with integrated environmental and clinical surveillance, specifically targeting bacteria isolated from plastic substrates, will help track the emergence and spread of novel AMR profiles, thereby providing an early warning system for public health.

## CRediT authorship contribution statement

**Michael J. Ormsby:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Luke Woodford:** Writing – review & editing, Methodology, Investigation. **James J. Mwesiga:** Writing – review & editing, Investigation. **Winnie Ernest:** Writing – review & editing, Investigation. **Dativa Shilla:** Writing – review & editing. **Daniel Shilla:** Writing – review & editing. **Richard S. Quilliam:** Writing – review & editing, Supervision, Project administration, Methodology, 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.127786.

## Data availability

Data will be made available on request.

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