

MICROPLASTICS AS POTENTIAL VECTORS OF ANTIMICROBIAL RESISTANCE IN HOSPITAL WASTEWATER: FROM RESISTOME DETECTION TO EVOLUTIONARY RISK

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Abstract

Antimicrobial resistance (AMR) in hospital wastewater develops within a highly heterogeneous environment. Along with resistant bacteria and antimicrobial resistance genes, these waste streams can contain mobile genetic elements, residual antimicrobials, disinfectants, heavy metals, and other chemical pollutants. The presence of microplastics adds another potentially important component to this system. Their surfaces can be colonized by microorganisms and support biofilm development, while the particles may also retain extracellular DNA and interact with chemicals present in the surrounding wastewater. These properties suggest that microplastics could alter the local conditions under which resistant microorganisms persist and resistance determinants are exchanged. Studies indicate that microplastic-associated biofilms can harbour antimicrobial resistance genes, virulence-associated determinants, mobile genetic elements, and prophage-associated sequences. Microplastic-associated biofilms may increase opportunities for horizontal gene transfer by promoting cell proximity and retention of extracellular genetic material.

Finding resistance genes on microplastics does not necessarily mean that they pose a clinical risk. What matters is whether the particles help resistant bacteria survive, exchange resistance genes, or maintain these genes in the wastewater environment. Microplastic-associated biofilms therefore deserve attention as active sites of resistance exchange. Current research is examining how pollutants, wastewater treatment, and microbial communities interact to shape these resistance patterns.

Current data suggest that Current evidence suggests that microplastics can provide surfaces that concentrate microorganisms, extracellular genetic material, and chemical

contaminants within localized biofilm-associated microenvironments. However, the extent to which these interactions increase clinically relevant antimicrobial resistance under environmentally realistic conditions remains uncertain. Future research should move beyond just finding new genes and instead focus on tracking real risks. This means studying how plastic properties, certain bacteria, gene movement, protein activity, and environmental persistence relate to actual clinical threats.

Keywords: antimicrobial resistance, hospital wastewater, microplastics, plastisphere, resistome, mobilome, horizontal gene transfer, genomic epidemiology, wastewater treatment, One Health.

1. Introduction

Antibiotic resistance is no longer limited to hospitals and clinical settings. Resistant bacteria and resistance genes are now being detected in many different environments, including wastewater systems, rivers and lakes, soil, food production, and other natural settings. Hospital wastewater is particularly important because it contains a complex mixture of antibiotics, resistant bacteria, resistance genes, strong disinfectants, pharmaceuticals, metals, free DNA, and other biologically active pollutants. Together, these conditions create an environment where bacterial selection, survival, close microbial contact, and genetic exchange can occur simultaneously.

Microplastics can act as surfaces on which wastewater microorganisms attach and grow. Once present in wastewater, these particles become coated with organic material and are gradually colonized by bacteria and other microorganisms. The resulting biofilm is often described as the plastisphere.

The importance of this plastic-associated environment has increased as accumulating evidence links microplastics to the dissemination of antimicrobial resistance. Experimental studies show that biofilms growing on plastic surfaces can increase uptake of extracellular DNA carrying resistance genes, while smaller, weathered plastic particles may promote substantially higher rates of gene transfer. Recent reviews have identified horizontal gene transfer, the accumulation of selective chemical compounds, and chemical co-selection as important mechanisms through which microplastics may contribute to the spread of antimicrobial resistance.

Recent research on hospital wastewater has advanced this field. A 2026 study combining incubation experiments, metagenomics, and genome reconstruction uncovered 68 resistance genes, 443 mobile elements, 414 virulence factors, and 129 prophages living right on colonizing microplastics. In addition, the researchers

reconstructed 360 metagenome-assembled genomes and found that mobile genetic elements accounted for a major share of the variation in those resistance genes. e.g. [2]

Nevertheless, an important conceptual problem remains. The presence of an ARG does not necessarily mean it is mobile or can be transmitted to another bacterial host. An ARG may be extracellular, incomplete, transcriptionally inactive, located in a non-mobile genomic region, or present in a microorganism with little opportunity to transmit to clinically relevant hosts.

ARG detection → bacterial host → MGE association → genetic mobility → expression → phenotypic resistance → persistence → successful transfer → clinical linkage

This framework distinguishes environmental resistome detection from actual evolutionary and epidemiological risk.

This review therefore aims not simply to summarise ARG abundance on microplastics, but to examine whether the plastisphere can alter the biological conditions that govern AMR persistence, mobility, and dissemination.

2. Hospital Wastewater and the Plastisphere as an Evolutionary Interface

Hospital wastewater brings together antibiotics, disinfectants, metals, resistant bacteria, and free DNA. Microplastics can become colonized by these bacteria and develop biofilms. The resulting matrix can keep cells, DNA, and chemicals in close proximity, which may favour the transfer of resistance genes between bacteria.

Three potential ecological roles can therefore be distinguished:

1. **Reservoir** — accumulation of pre-existing ARGs and resistant organisms.
2. **Selective niche** — enrichment of microorganisms able to tolerate local chemical and ecological conditions.
3. **Evolutionary interface** — modification of the physical and chemical conditions that facilitate genetic exchange.

Evidence for the reservoir and selective-niche roles is currently stronger than evidence for a general evolutionary-amplification effect. The distinction is critical. If microplastics merely concentrate organisms that are already present in wastewater, their ecological role differs fundamentally from a situation in which they independently increase HGT or the emergence of resistant phenotypes.

Recent reviews suggest several plausible mechanisms for AMR selection within the plastisphere, including increased cell proximity, retention of extracellular DNA and adsorption of antimicrobial or co-selective compounds.

Researchers need to determine whether ARGs behave differently in microbial communities growing on microplastics compared with those growing without them.

3. Microplastic Aging as an Emerging AMR Determinant

Microplastics do not remain chemically or physically unchanged after entering wastewater. Environmental aging can produce oxidation, fragmentation, changes in surface roughness, altered surface charge, and changes in functional groups. These modifications can influence microbial attachment, biofilm development and contaminant adsorption.

Importantly, a particle's environmental history may therefore be more informative than its polymer identity alone.

A useful experimental classification is:

pristine particle → wastewater-exposed particle → aged particle → fragmented particle

These stages should be evaluated for:

- microbial colonisation;
- biofilm succession;
- ARG abundance;
- ARG–MGE association;
- extracellular DNA retention;
- bacterial host composition;
- HGT frequency;
- resistance phenotype.

Experiments already show that a plastic particle's age and size can strongly affect how easily resistance genes are transferred. For instance, one study found that smaller, weathered microplastics increased gene transfer frequencies much more than larger, brand-new plastic particles.

A 2024 review further proposed that photoageing can increase surface functional groups and sorption capacity, potentially modifying interactions between antibiotics, ARGs and microbial communities.

However, increased adsorption does not automatically mean increased biological risk. A chemical compound adsorbed to a particle must remain sufficiently bioavailable to exert selective pressure. Similarly, increased ARG adsorption does not necessarily translate into successful genetic transfer.

Therefore, aging research should move beyond adsorption measurements and ask whether aging changes the **probability of functional resistance transfer**.

4. Spatial Structure of the Plastisphere

A major limitation of conventional plastisphere research is its reliance on bulk community measurements.

A mature biofilm is spatially heterogeneous. Oxygen, nutrients, pH, redox potential and contaminant concentrations can differ over very small distances. Consequently, bacterial populations occupying different microenvironments on the same particle may experience substantially different physicochemical conditions.

This spatial organization may influence AMR in at least three ways.

First, localized high cell density can increase donor–recipient contact.

Second, extracellular polymeric substances may retain DNA and modify its accessibility.

Third, chemical conditions may vary across the particle surface, creating small areas where resistant bacteria have a selective advantage. Gene transfer may therefore be more common in some parts of the biofilm than others. Future studies should combine metagenomic data with:

- confocal microscopy
- single-cell genomics
- fluorescence-based localization
- microscale chemical mapping
- spatial transcriptomics where technically feasible.

The critical objective is to determine whether **ARG-bearing organisms, recipient cells and MGEs spatially converge** within the same microenvironments.

This would provide stronger mechanistic evidence than bulk ARG abundance alone.

5. From Resistome to Mobilome

The most important conceptual limitation of ARG-centred studies is that **gene abundance does not equal gene mobility**.

An ARG may be abundant but genetically isolated. Conversely, a low-abundance ARG located on a highly mobile plasmid in a clinically relevant bacterial host may represent a greater evolutionary concern.

An ARG count does not show its host or genetic location. Microplastic-associated communities can contain resistance genes within different bacterial and mobile-element backgrounds. Linking these components requires methods such as long-read sequencing, genome reconstruction, and Hi-C analysis.

Therefore, **culture-based validation, conjugation assays, transformation experiments and phenotypic antimicrobial susceptibility testing** remain important where feasible.

A particularly useful future endpoint would be the identification of ARGs that are simultaneously:

1. present
2. associated with a bacterial host
3. linked to an MGE
4. expressed
5. phenotypically functional
6. transferable
7. persistent under environmentally relevant conditions.

6. Prophages: An Understudied Route of Resistance Mobility

Most environmental AMR studies have concentrated on plasmids and integrons. Phages and prophages have received much less attention, despite their potential role in resistance-gene transfer.

That picture is changing. A recent hospital wastewater study identified 129 prophage-associated sequences within microplastic-associated microbial communities. In addition, independent large-scale analyses of hospital wastewater have shown a wide variety of phages, found resistance genes linked directly to them, and mapped potential phage-and-host connections.

Finding prophage DNA is not enough to confirm that phages are transferring resistance genes. Experiments are needed to test phage activation, resistance-gene packaging, release of phage particles, and transfer after treatment.

Resolving these questions matters because plasmid-mediated mechanisms alone may not capture the full range of genetic pathways driving environmental AMR dissemination.

7. Chemical Co-selection in the Plastisphere

- **potential enhancement of horizontal gene transfer within dense biofilms**
- **concentration of chemicals capable of selecting or co-selecting for resistance**
- **retention or gradual release of plastic-associated chemical additives**

Experimental studies have also found complex interactions among heavy metals, disinfectants, and resistance genes within these plastisphere communities.

However, the interpretation requires caution.

Total chemical burden and microbial exposure are not equivalent. Selection depends on the fraction of a compound that remains accessible to microorganisms and is present at a biologically active level.

Therefore, future studies should examine environmentally realistic mixtures such as:

antibiotics + disinfectants + metals + pharmaceuticals + organic contaminants

and measure:

- gene expression
- ARG enrichment
- MGE abundance

- HGT frequency
- microbial succession
- resistance phenotype
- bacterial fitness.

Mixture experiments should also test antagonistic and synergistic interactions because environmental chemical effects are not necessarily additive.

8. Wastewater Treatment: Are We Destroying Resistance, or Just Moving It Around?

Looking only at the liquid effluent does not give a complete picture of what happens to ARGs during wastewater treatment. A decrease in ARGs in the water phase may not mean that they have been destroyed. Some may instead become associated with suspended solids, sludge, or microbial communities growing on microplastics. For this reason, studies of ARG removal should consider the water, particulate matter, microplastic-associated biomass, and sludge together. This can help determine whether resistance determinants are actually degraded or simply removed from the water and redistributed elsewhere in the treatment system.

raw influent → biological tanks → disinfection → sludge → treated discharge

9. The Phenotype Gap: Finding DNA Doesn't Mean Finding Trouble

Most environmental AMR studies rely heavily on molecular detection of resistance-associated DNA in wastewater, sludge, and associated particulate compartments. However, DNA detection alone does not establish functional resistance.

DNA detection $\neq$ gene expression $\neq$ resistance phenotype

Genomics — identification of resistance-associated genetic determinants and their genomic context.

Transcriptomics — assessment of resistance-gene transcription and functional activity.

Culture-based and phenotypic testing — determination of antimicrobial susceptibility and functional resistance.

A stronger framework combines:

metagenomics + metatranscriptomics + culture-based susceptibility testing

where feasible.

This is especially important for novel ARGs whose clinical relevance cannot be established through sequence similarity alone.

For a resistance determinant to be considered high-confidence environmental AMR evidence, ideally there should be evidence of:

sequence → host → mobility → expression → phenotype

10. The Environmental-to-Clinical Transmission Gap

The presence of ARGs on microplastics does not establish a clinical connection. Evidence is still needed to identify the bacterial hosts, confirm gene activity and transfer, and trace the same resistance determinants between environmental and clinical isolates.

11. Particle-Specific AMR Risk

Treating all microplastics as one exposure category may obscure important biological differences.

Relevant characteristics include:

Polymer composition	Surface chemistry and microbial attachment
Particle size	Surface-to-volume ratio and cell concentration
Ageing	Surface transformation and contaminant adsorption
Roughness	Biofilm attachment and spatial organization
Biofilm maturity	Cell proximity and genetic exchange
Chemical loading	Local selective pressure
Morphology	Hydrodynamic behaviour and colonization

Experimental evidence indicates that polymer characteristics, size, and aging can influence transformation and ARG-associated processes.

Therefore, future risk assessments should develop **particle-specific AMR profiles** rather than treating all microplastics as biologically equivalent.

A particle with high surface roughness, advanced aging, mature biofilm, and substantial chemical loading may have a different ecological function from a pristine particle with limited colonization.

12. Establishing Causality

Methodological Challenge

Wastewater studies alone make it difficult to determine whether microplastics drive changes in resistance or simply provide another surface on which the existing microbial community can accumulate. A useful way to address this problem would be to examine the same microbial system without particles and after exposure to pristine microplastics, aged microplastics, and non-plastic particles. Alongside ARG abundance, the analysis should consider transcriptional activity, ARG linkage to mobile genetic elements, HGT, antimicrobial susceptibility, bacterial fitness, and persistence.

It is still unclear whether microplastics have a role in AMR beyond that of ordinary particles found in wastewater. Bacteria can colonize almost any suitable surface, so the presence of a plastisphere alone is not evidence of a unique resistance effect. The comparison becomes more informative when microplastics and natural particles are exposed to the same wastewater conditions and examined for differences in contaminant binding, microbial composition, and gene transfer. Such experiments would help determine whether the plastic surface itself changes resistance dynamics or simply provides another place for microorganisms to accumulate.

13. Priority Future Research

Priority Areas for Future Research

Several areas require further investigation before the contribution of microplastics to environmental AMR can be clearly established. First, studies should examine whether aging changes microplastics' ability to support ARG transfer, rather than simply increasing microbial attachment or adsorption of genetic material. Comparing pristine and environmentally aged particles under similar conditions would help resolve this question.

A second priority is to improve the resolution of ARG–MGE–host relationships. Identifying which resistance genes are physically associated with mobile genetic

elements and which bacterial hosts carry them would provide a more meaningful indication of their potential for dissemination than ARG abundance alone.

The possible contribution of prophages also deserves greater attention. Detecting prophage-associated resistance genes does not demonstrate active transduction, and experimental studies are needed to determine whether these elements can contribute to resistance transfer within plastisphere communities.

The spatial organization of the plastisphere is another important area for study. Microscale measurements could help determine whether local conditions such as nutrient availability, chemical gradients and high cell density bring potential donor and recipient bacteria into closer contact and thereby influence HGT.

Chemical interactions should also be investigated under environmentally relevant conditions. Antibiotics, disinfectants, metals and other contaminants may occur together in hospital wastewater, and their combined effects on resistance selection, microbial community structure and bacterial fitness may differ from their individual effects.

Wastewater-treatment studies should follow resistance determinants through the entire treatment process, including water, microplastic and sludge compartments. Tracking ARGs together with their bacterial hosts and associated MGEs would help distinguish actual degradation from physical removal or redistribution.

An ARG identified by sequencing is only a genetic observation. Its biological relevance requires evidence that the gene is transcribed and associated with a resistance phenotype. Researchers can explore a possible link to clinical AMR by comparing environmental samples with patient-derived or healthcare isolates for matching resistance variants and their surrounding plasmid or mobile-element sequences. A genetic match would strengthen the association, but would not establish that the gene moved from the environment into a patient.

The same limitation applies to microplastic-associated AMR. An ARG count says little about the biological context in which the gene occurs. Relevant factors include the particle type, its colonizing organisms, the genetic location and mobility of the ARG, its persistence and expression, and the pathogenic characteristics of its host. Taken together, these data are needed to determine whether microplastics are simply accumulating resistance determinants or are associated with conditions that favour their dissemination.

14. A Next-Generation Research Framework

A comprehensive investigation of plastsphere-associated AMR can be organized into five analytical levels.

Level 1 — Particle ecology

Polymer, size, morphology, ageing and surface chemistry.

Level 2 — Plastsphere structure

Biofilm development, microbial succession and spatial organization.

Level 3 — Resistance architecture

ARGs, MGEs, prophages, bacterial hosts and virulence determinants.

Level 4 — Functional validation: Assessment should include gene expression, horizontal gene transfer, antimicrobial susceptibility, bacterial fitness, and persistence.

Level 5 — Clinical linkage: Compare environmental findings with contemporaneous clinical isolates using high-resolution genomic epidemiology.

An ARG measurement does not reveal its biological behaviour. The same gene may be inactive in one host and clinically relevant in another, depending on its expression, genetic context, mobility, and persistence. Functional assays and genomic analyses are needed to resolve these differences. Risk assessment should consequently give greater weight to resistance determinants supported by evidence of activity, transfer, persistence, and clinical relevance.

15. One Health Implications and Environmental Infection Prevention

Hospital wastewater provides an important connection between infection control within healthcare facilities and the environmental dimensions of AMR. Once discharged from healthcare facilities, wastewater carries a complex mixture of resistant microorganisms, resistance determinants, antimicrobial compounds, disinfectants, and other chemical residues into environments where these biological and chemical pressures interact with established microbial communities before and during treatment. Microplastics add another component to this environment because they can provide persistent surfaces for microbial colonization and may carry associated microorganisms and genetic material through different stages of the wastewater system.

This relationship can be considered along a broader pathway:

Healthcare facility → hospital wastewater → treatment system → receiving environment → environmental microbial communities → potential human and animal exposure

Viewing the problem in this way places the hospital-wastewater platisphere within a One Health context. AMR arising in clinical settings does not necessarily remain confined to the hospital, and resistance detected in the environment cannot be understood independently of its possible human, animal, and healthcare sources. Wastewater therefore represents an important interface where these compartments meet.

Microplastics are only one part of the AMR environment found in hospital wastewater. The same wastewater carries antibiotic residues, resistant bacteria, extracellular DNA, disinfectants, metals, and other pollutants, all of which can alter microbial survival and selection. Reducing the plastic fraction alone would therefore leave many of the processes involved in resistance persistence and dissemination unchanged.

Reducing microplastics addresses only one part of the wastewater AMR problem. Resistance is also shaped by antibiotic exposure, hospital infection-control practices, sanitation, pharmaceutical residues, treatment performance, and the organisms released with treated waste. These factors interact along the route from the healthcare facility to the receiving environment. Monitoring resistance at several points along this route can therefore show where selection or release is occurring and where intervention is most needed.

Hospital wastewater can consequently be viewed as part of a wider environmental infection-prevention system. Monitoring this compartment may provide information not only on the release of resistant organisms and resistance determinants from healthcare facilities, but also on their persistence and redistribution during treatment. Integrating wastewater surveillance with clinical and environmental data could therefore provide a more complete picture of how AMR moves across interconnected One Health compartments.

16. Limitations of the Current Evidence

Despite rapidly increasing research interest, several limitations prevent definitive conclusions regarding the clinical importance of microplastics in AMR dissemination.

First, many studies remain observational or based on laboratory systems that may not reproduce the chemical, microbial and hydrodynamic complexity of hospital wastewater.

Second, ARG abundance is frequently used as the primary outcome even though abundance does not demonstrate mobility or phenotypic resistance.

Third, metagenomic association between an ARG and an organism does not necessarily demonstrate that the organism carries a functional, transferable resistance determinant.

Fourth, laboratory transformation or HGT experiments may use concentrations, particle sizes or chemical conditions that differ from environmentally realistic exposure.

Environmental studies have detected ARGs and resistant bacteria on microplastics, but clinical relevance has not been established. The missing evidence is a demonstrated link between plastisphere-associated resistance and resistance occurring in patients or healthcare settings. Until that link is shown, the clinical implications of microplastic-associated AMR should remain an open research question.

17. Conclusion

Microplastics are part of the microbial and chemical environment of hospital wastewater, where resistance genes and resistant bacteria can accumulate. Their presence alone does not show that they make resistance spread more efficiently than other particles. The unresolved question is whether the plastisphere has a measurable effect on the behaviour and movement of resistance genes.

This can only be addressed by comparing microplastics with relevant non-plastic particles and examining the organisms, genetic elements, activity, and persistence involved. Clinical genomic data may provide additional evidence about the relevance of environmental findings. Until these links are demonstrated, microplastics should be considered a possible component of environmental AMR rather than a proven cause of clinical resistance.

For now, microplastics should be viewed as one part of the wider wastewater resistome. Their contribution to AMR will become clearer only when ecological observations are supported by functional and epidemiological evidence.

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