Review

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Review

Antibiotic Resistance Gene Enrichment on Plastic Wastes in Aquatic Ecosystems and Fishery Products

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Abstract

This comprehensive review gathers the current knowledge on the link between plastics wastes and antibiotic resistance genes (ARGs) selection and transmission in aquatic ecosystems that can lead to the ARG contamination of fishery products, a relevant source of MPs introduction in the food chain. Indeeed, plastic debris in aquatic environments aare covered by a biofilm (plastisphere) in which antibiotic-resistant bacteria (ARB) are selected and the horizontal gene transfer (HGT) of ARGs is facilitated. The types of plastic wastes considered in this study for their role in ARG enrichment are mainly microplastics (MPs) but also nanoplastics (NPs) and macroplastics. Studies regarding freshwaters, seawaters, aquaculture farms and ARG accumulation favored by MPs in aquatic animals were considered. Most studies were focused on the identification of the microbiota and its correlation with ARGs in plastic biofilms and a few evaluated the effect of MPs on ARG selection in aquatic animals. An abundance of ARGs higher in the plastisphere than in the surrounding water or natural solid substrates such as sand, rocks and wood was repeatedly reported. The studies regarding aquatic animals showed that MPs alone or in association with antibiotics favored the increase of ARGs in the exposed organisms with the risk of their introduction in the food chain. Therefore, the reduction of plastic pollution in waterbodies and in aquaculture waters could mitigate the ARG threat. Further investigations focused on ARG selection in aquatic animals should be carried out to better assess the health risks and increase the awareness on this ARG transmission route to adopt appropriate countermeasures.

Keywords: plastics wastes; aquatic environments; antibiotic residues; plastisphere; antibiotic resistance genes; fish; seafood

1. Introduction

According to the first global and regional estimate of antimicrobial resistance (AMR) mortality, in the years 1990 to 2021 deaths directly attributable to the drug resistance of an infection or associated with a drug-resistant infection increased by more than 25 000 annually with methicillin-resistant *S aureus* (MRSA), multidrug-resistant tuberculosis, carbapenem-resistant *Klebsiella pneumoniae*, and carbapenem-resistant *Acinetobacter baumannii* as the bacteria most often implicated. It was also estimated that until 2050, 39·1 million deaths attributable to AMR and 169 million deaths associated with AMR may occur for people aged 70 years and older [1].

Plastic wastes represent a widespread pollutant in the environment deriving from multiple sources among which agriculture and food production with almost 50 million tonnes of plastic used for cultivations, animal husbandry, fishery, forestry, and food packaging in 2019 foreseen to rise to 9.5 million tonnes in 2030 globally [2]. The main sources of plastic wastes in aquatic ecosystems derive from wastewater treatment plants (WWTPs), irrigation with wastewater, sludge fertilization and landfill leachates reaching surface and groundwater [3].

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According to the fragment size plastic materials are classified as macroplastics (>2.5 cm), mesoplastics (between 0.5 and 2.5 cm), microplastic or MPs (<5 mm) and nanoplastics or NPs (<100 nm) [3,4]. MPs of polymers such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene therephthalate (PET), polyvinyl chloride (PVC) originate from the direct emission in the environment of microbeads, or from the chemical or mechanical fragmentation of larger plastic debris. These constitute a major component of marine litter, defined as any solid waste of human origin discarded in the sea or which reaches the sea by dispersion from other environments [5,6].

The occurrence of MPs in form of textile microfibers or other shapes in the gastrointestinal tract of marine fish and seafood species intended for human consumption was demonstrated in numerous investigations so these food sources pose a high risk of MP introduction in the human diet. Indeed, small fishes and seafood are consumed without previous evisceration and it was also shown that fish evisceration does not prevent the presence of MPs in edible tissues [5,7,8]. MPs were detected in aquatic organisms such as fishes, crustaceans, molluscs, algae, corals, and marine mammals in at least 57 countries and regions and their concentration was found to increase from zooplankton to fishes. Benthic crustaceans and molluscs can contain hundreds or several thousand MP particles, respectively, that derive both from sediment and water. These are ingested or adsorbed by respiration by fishes and can even be transferred to eggs. The amounts of MPs in fishes depend on the level of local pollution, size of the fish, type of feeding, rapidity of movements and depth inhabited by the species. Aquaculture fishes were found to ingest higher amounts of MPs than wild fishes from the same area and mostly as fibers since these are not retained by the water filtration system. In addition, MPs in these systems originate from the plastic made equipment [9].

The absorption of antibiotics by MPs in aquatic environments started to be investigated after observing that sulfonamides, trimethoprim and fluoroquinolones are stable in water and that MPs can transfer organic pollutants to the food web of aquatic organisms. It emerged that ciprofloxacin, amoxicillin and tetracycline are adsorbed on polyamide (PA) by hydrogen bond formation and that ciprofloxacin, amoxicillin, trimethoprim, sulfadiazine and tetracycline absorbed at levels that followed the order PP > PS > PE > PVC. The adsorption on MPs was more efficient in freshwater than in seawater since both antibiotics such as ciprofloxacin and amoxicillin and MPs are negatively charged in this environment [10].

Plastic wastes dispersed in seawater and freshwater harbor a specific biofilm microbiota denominated “plastisphere”, a term coined after that scanning electron microscopy (SEM) showed the presence of a varied microbial consortium on plastic debris filtered from the North Atlantic ocean. This biofilm comprised also pathogens of the genus *Vibrio* as shown by High throughput sequencing (HTS) of the small rRNA gene subunit [11]. Biofilm formation occurs through the phases of adsorption of microorganisms on the plastic surface, formation of multilayered clusters of bacterial cells and development of a three-dimensional network [3]. Biomass growth on plastic particles is favored by the so called “ecocorona” resulting from the accumulation on their surface of organic and inorganic materials that facilitate the colonization by microorganisms [12]. Plastic biofilms can contain up to 100 - 5000 folds higher loads of antibiotic-resistant bacteria (ARB) compared to the surrounding water [3,13] since antibiotic adsorption implies a selective pressure that favors the predominance of ARG bacterial carriers [14]. Mobile genetic elements (MGEs) implicated in ARG horizontal gene transfer (HGT) and detected in the plastisphere include the class 1 integron, gene cassette capture elements involved in ARG spread and encoding the integrase *intI*1 and plasmids pBB55, pGS05 and the conjugative plasmid RP4 that encodes resistance to kanamycin, tetracycline, and ampicillin [4,14].

Biofilm development is favored by plastic degradation caused by exposure to UV rays, aging, oxidation and thermal stresses that increase surface roughness and favor bacterial colonization. Size, shape and color were shown to influence the biofilm development on MPs. Biofilms alter the morphology, hydrophobicity, surface area and functional groups in MPs affecting their capacity to adsorb chemical pollutants [14]. For example, photoaging in water led to an increase of high energy binding sites and increased adsorption of tetracycline on PVC MPs and in the biofilm formed in a

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suspension of activated sludge the abundance of microorganisms and the abundance of the tetracycline ARGs *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetK* determined by qPCR was higher on aged MPs [15].

In one of the first studies the MP-associated microbiota was found to vary in composition according to nutrient availability, salinity and proximity to the coast and differed between PE and PS. Bacterial families enriched in MPs in all coastal stations were Hypomonadaceae and Erythrobacteraceae. Hypomonadaceae produce strongly adhering polysaccharides and present cell appendages called prosthecae that allow the uptake on nutrients from a vast area. Therefore, these bacteria are the first to adhere even to smooth MPs. Erythrobacteraceae are abundant on MPs because they degrade the polycyclic aromatic hydrocarbons (PAHs) adsorbed on plastic. In addition, the potentially pathogenic genus *Tenacibaculum* of the family Flavobacteriaceae was detected both on PE and PS. The genus *Sphingopyxis* of the family Sphingomonadaceae was abundant on MPs in a WWTP and found to harbor a Class I integron encoding ARGs leading to the hypothesis that MPs represent a hotspot for ARG HGT [16]. Indeed, it was shown that the high bacterial density in MP biofilms facilitates ARG transfer by the main three routes, i.e. natural transformation, conjugation and transduction [14]. Therefore, the detrimental effects on health of MP ingestion by humans and animals, that are oxidative stress, cytotoxicity, chronic inflammation, neurologic and immune disorders, and toxicity caused by the release of chemicals including bisphenol A (BPA), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides [5], can be further exacerbated by their potential to disseminate AMR.

The purpose of this comprehensive review was summarizing the updated evidences on the involvement of plastic debris in ARG and multidrug resistance genes (MDRGs) selection, increase and HGT in aquatic environments and of their transmission to aquatic organisms, among which species used as food. The scientific articles regarding these topics were retrieved from Google Scholar (https://scholar.google.com/schhp?hl=it, accessed on 24 September 2025), and EMBASE (https://www-embase-com.bibliosan.idm.oclc.org/ accessed on 24 September 2025) with two search strings, namely “antibiotic resistance AND microplastics AND water” (string 1), and “antibiotic resistance AND microplastics AND fish” or with the word “mollusc” or “mollusk”, or “crustacean”, or “shellfish”, or “seafood” or “sea food” in place of “fish”(string 2). The searches were sorted by relevance in GoogleScholar without time restriction and article screening was continued until two consecutive pages did not provide further relevant publications. The scientific articles considered in this study were selected among the first 770 retrieved from GoogleScholar and the 283 articles retrieved in Embase with the search string 1. With the search string 2 with all its variants the first 50 articles retrieved in GoogleSchola were considered while in Embase 12 articles were retrieved with the word "fish", 9 with the words "sea food", 4 with the word "seafood", 4 with the word "crustacean" and 3 with the word "mollusc". A first selection was done by title, a second selection by abstract content and a third selection by whole text content. The articles finally considered were those documenting the role of plastic wastes in selection and spread of ARGs and MDRGs in aquatic species habitats. Special attention was dedicated to the correlations established between the microbiota in the plastisphere and ARGs and to the factors found to influence their composition. Therefore, also articles elucidating the physiological mechanisms that favor ARG HGT in the plastisphere were commented. Both non-biodegradable (NBPs) and biodegradable plastics (BDPs) were taken into account and NPs and macroplatics were also considered as substrates involved in ARG selection and transmission. Throughout the text the indicated ARG gene functions are those defined by the Comprehensive Antibiotic Resistance Database (CARD, https://card.mcmaster.ca/home, accessed on 20 July 2025) and the nomenclature of the miicroorganisms was that used in the original source. The currently valid names can be found at https://lpsn.dsmz.de/ (accessed on 30 September 2025). The studies cited in each section are ordered chronologically to highlight the evolution of investigation methods and the progression of research achievements.

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2. Antibiotic Adsorption on Plastic Polymers

The adsorption of environmental antibiotic residues to plastic debris drives their colonization by ARB and investigations regarding antibiotic binding contribute to explain the ARG selection dynamics. Adsorption isotherms were initially used to this aim by using antibiotic concentrations in the range 0.2 – 50 mg/L that are much higher than those found in the environment, that are of the order ng/L or µg/L. Adsorption isotherms showed that aged plastics have an increased adsorption capacity and that the interactions favoring the binding of substances to plastics are *i* electrostatic, i.e. dependent on the electrical charges of the polymer and the antibiotic, *ii* hydrophobic, i.e. occurring between non-polar plastics such as PS, PE, PP, and PET and antibiotics at a rate dependent on the hydrophobicity of the latter, *iii* of Van der Waals and π-π type for aliphatic polymers, such as PVC and PE and aromatic polymers, such as PS, and *iiii* pore-filling with greater pores in plastic debris adsorbing an higher number of antibiotic molecules. Moreover, antibiotic adsorption increases as the MP particle size decreases because of the greater surface/volume ratio. For this reason, aging of MPs caused by UV rays, mechanical shearing and exposure to extreme temperatures increases their adsorption capacity. Parameters such as pH, presence of electrolytes, dissolved organic matter and competing contaminants influence antibiotic adsorption rate on MPs [17].

Recently, machine learning (ML) was applied to obtain generalizable prediction models for the adsorption of different antibiotics to plastic materials. One of these models is based on data of poly-parameter Linear Free-Energy Relationships (ppLFER) molecular descriptors based on Quantitative Structure Property Relationship (QSPR) obtained from scientific publications on single adsorbents and adsorbates and combines the algorithms tree-based random forest (RM), eXtreme Gradient Boosting (XGB) and Light Gradient Boosting machine (LGBM). The characteristics considered for the adsorbents in the model are the specific surface area (SSA), that determines the number of active sites, the carbon percentage (C%), that determines surface homogeneity and influences the affinity for the adsorbates as dependent on hydrogen bonds and Van der Waals forces, the H to C ratio that determines the aromaticity of MPs and their interaction with aromatic antibiotics and the O to C ratio that indicates the presence of hydroxyl, carboxyl and carbonyl groups and the tendency to interact with ionic antibiotics. For the antibiotics the Abraham descriptors correlating structure and properties in different conditions were taken into account. The output parameter was the logarithm of the solute equilibrium partitioning coefficient "Kd". The best fitting model allowed to maximize the coefficient of determination (R^2) and minimize the Root Mean Square Error (RMSE). The SHapley Additive exPlanation (SHAP) method allowed to identify as top adsorption predictors the hydrophobicity of the antibiotic, the polymer surface polarity, the fraction of ionized species at the test pH and the molecular volume [18].

The molecular dynamics (MD) approach to the study of antibiotics-MP interactions represents another alternative to the experimental methods to elucidate the antibiotic adsorption dynamics in MPs and define how these contribute to their transport and persistence for risk assessment and remediation monitoring. Amoxicillin, tetracycline and ciprofloxacin were used as model molecules, 50 repeat units of PP and PS as model MPs and a model water with added NaCl ions as natural environment in computational simulations of all the possible molecule interactions based on the molecular structures. Oxidative aging was simulated by introducing (-OH) and carbonyl (-C=O) groups. This method predicted the adsorption enhancement by aged MPs due to the increased roughness and introduction of polar groups. It was shown that PS particularly when aged, adsorbs the antibiotics more efficiently than PP for its higher hydrophobicity and π-π interactions with aromatic antibiotics but PP also efficiently binds the antibiotics after aging due to the increased polarity. This model indicated that MPs prolong the persistence of antibiotics in aquatic environments. The root-mean square deviation (RMSD) analysis demonstrated the structural stability of molecules during the adsorption process [19].

In addition to the antibiotics, plastic polymers adsorb extracellular DNA particularly in conditions of high ionic strength that reduce electrostatic repulsion. Among PE, PET and PS MPs,

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PET showed the highest adsorption capacity for linear DNA possibly facilitated by the ester groups and adsorption occurred in a short time (30 min) [20].

3. Demonstrations of ARG HGT in the Plastisphere

Studies demonstrating ARG HGT by natural transformation or conjugation in MP biofilms or in presence of NPs were carried out in controlled conditions using model bacteria. It was shown that *E. coli* DH5α exposed to PS NPs was transformed with increased frequency with the plasmid pUC19 encoding ampicillin resistance, produced dose-dependent amounts of reactive oxygen species (ROS), as determined with the fluorescent dye H2DCF-DA, and showed increased catalase (Cat), superoxide dismutase (Sod) and glutathione peroxidase (GSH-Px) activities. Addition of the ROS scavenger thiourea reversed ROS formation and decreased the transformation frequency, thus confirming that oxidative stress favored transformation. Moreover, NPs induced an increased expression of the secretion system genes *sec*A, *sec*B, of the arginine translocase gene *tat*A and the gene *hof*B involved in the synthesis of the type II/IV conjugative pilus [21].

A strain of *Bacillus subtilis* and a strain of *Acinetobacter baylyi*, naturally competent bacteria, were allowed to form biofilms in a mineral solution on PE, PP and PS MPs in presence of plasmid pUC19 and the non-conjugative plasmids pRK415, encoding tetracycline resistance and the green fluorescent protein gene *gfp* for the identification of tranformants by fluorescence microscopy. The frequency of transformation for both plasmids was higher in the MP biofilms than in sand used as control natural substrate (NS) and in water and its extent followed the order PP > PE > PS with PP showing the highest bacterial density. The transformation frequency was higher in MPs of 300 μM size compared to those of 3 mm size and on MPs aged by exposure to UV light. Transcriptome analysis showed that on PS and PP biofilms, the flagellum movement genes *motA*, *glgA*, and *bssR* and the quorum sensing (QS) gene *lsr*K were upregulated in *B. subtilis*, the genes involved in the synthesis of polysaccharides *pga*A and *pga*B were upregulated and biofilm formation genes, *com*A and *pli*X slightly upregulated in both bacteria in smaller PP particles with a higher expression level on aged particles. In a multi-species microcosm in river water, the transformation efficiency of pRK415 was higher in the MP biofilms than in the NS at some time intervals and the transformants identified by 16S rRNA gene sequencing belonged to the species *B. cereus*, *Paraclostridium* spp. and *Enterococcus faecalis*. The latter species was detected only in MPs [22].

The role of QS in ARG transfer by transformation in MP biofilms was demonstrated in *B. subtilis* CGMCC 1.286 that produces acyl-homoserine lactones (AHLs) QS signal molecules, including C8-HSL, 3-oxo-C10-HSL, and C12-HSL. Their exogenous addition at concentrations varying from 0 to 10,000 ng/L led to a dose-dependent increase of extracellular polysaccharides (EPS) synthesis and transformation frequency of pRK415 in biofilms formed on PS, PE and PP MPs in mineral solution. This was of the order of 10^{-4}, higher on MPs than on NS, since the bacterial density in the MP biofilms was about one hundred-fold higher. The QS inhibitors 2(5 H)-furanone, furaneol, coumarin, and benzpyrole led to the decrease of EPS production and transformation efficiency. AHL presence led to the upregulation of the competence genes *com*X and *com*A, of the environmental DNA uptake gene *rec*O, and of the EPS production genes *tasA*, *epsG*, and *tapA*. The latter were downregulated upon exposure to QS inhibitors [23].

A frequency of plasmid transfer by conjugation three orders of magnitude higher than in water was observed in PS MPs in a microcosm containing water from Lake Stechlin (Germany) in which the *E. coli* MG1655 donor chromosomally tagged with the red-fluorescence expressing cassette *lac*Iq-Lpp-mCherry-km^R, and harboring the self-transmissible trimethoprim resistance plasmid pKJK5 tagged with *gfp*, and a *Pseudomonas* spp. recipient isolated from the lake were incubated. The *lac*I repressed *gfp* in the plasmid donor so this could be expressed only in the transconjugants that were revealed by green fluorescence using flow cytometry and confocal microscopy. Bacteria detached with two methods from PS MPs deployed for four weeks in the lake received plasmid pKJK5 in mating experiments with the *E. coli* donor with one order of magnitude higher transfer frequency than free-living bacteria isolated from the lake water. Metataxonomy showed that the microbiota

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comprised mainly Gammaproteobacteria, Actinobacteria and Betaproteobacteria but with a different genera distribution between MPs and water and that *Arthrobacter* was the genus transformed vith highest frequency [24].

The same donor/recipient system was used to demonstrate that tire wear particles (TWP) of 10/100 μm size constituted by styrene-butadiene rubber supported conjugation particularly at 1:10 and 1:100 donor/recipient ratio. Indeed, the bacteria colonized the TWPs. The transfer frequency was 27-fold higher than in suspension and in PS MPs. HGT was enhanced by TWPs also when a lake water bacterial community was tested as recipient [25].

SEM showed that the red fluorescent donor *E. coli* MG1655 mentioned above and the recipient *E. coli* ATCC 25922, or recipient sludge bacteria, incubated in LB broth at 37°C in presence of 0 to 500 mg/L on a polydimethylsiloxane (PDMS) membrane chip formed a biofilm on PS particles with a diameter greater than 2 μm while particles of 0.2 μm size aggregated on the bacterial cell surface. At MP concentrations lower than 200 mg/L the 0.2 μm particles promoted conjugation and at higher MP concentrations also the larger particles. The plasmid transfer to sludge bacteria was maximum on particles of 15 μm/20 μm at the concentration 500 mg/L that induced the upregulation of the mating genes *trb*Bp and *tra*F and the DNA transfer and replication genes *trf*Ap and *tra*J in *E. coli*. The 0.2 μm particles increased cellular ROS and membrane permeability. The contact of transconjugants with MPs was demonstrated by complementation of fluorescence observation with clear field images and taking into account the theoretical geometric contact probability [26].

The *E. coli* HB101 donor strain harboring plasmid RP4 and the recipient strain *E. coli* NK5449 resistant to rifampicin showed no significant variation in the expression of genes related to oxidative stress in biofilms formed in 4 mm PP, PS, PVC, and PE particles in simulated estuarine water and controlled nutrient concentration. However, a 4 to 11-fold increased expression of the outer membrane protein genes *omp*A, a pathogenicity factor involved in adhesion and invasion, *omp*C, and *omp*F that form osmo-protection and antibiotic resistance channels and a 1 to 5-fold increased expression of *tra*G involved in the formation of the mating apparatus, conjugative pilus and DNA transfer occurred at a higher extent in PE biofilms [27].

A theoretical investigation with *E. coli* as donor and *Pseudomonas aeruginosa* as recipient indicated that the average binding energy with the predicted active codons of *E. coli* plasmid harboring multiple ARGs for enzymes involved in HGT, i.e. the relaxase, the type IV secretion system (T4SS) coupling protein (T4CP) and its component VirB5, and the DNA ligase of *P. aeruginosa*, increased by 1.12 to 2.02 folds in presence of PP, PVC, PET, PE, PS, and polycarbonate (PC), in agreement with previous evidence that VirB5 is upregulated in presence of PE and PVC MPs. The multiple criteria decision making (MCDM) method VIKOR indicated PP as the material with the highest risk of HGT. The GROningen MAchine for Chemical Simulations (Gromacs, https://www.gromacs.org/ accessed on 12 April 2025) biomolecule interaction prediction software indicated that PP stress increased by 3 to 4-fold the number of hydrogen bonds between the active codons and the relaxase and by 8 to 10-fold those with VirB5. The latter increased also upon exposure to PC [28].

Conjugative transfer occurred between the donor strain *P. putida* KT2440::*lac*Iq-dsRed, harboring the conjugative plasmid *gfp*-RP4 and the recipient strain *E. coli* NK5449 in LB medium in the presence of PVC and PS MPs. Laser scanning confocal microscopy (LSCM) allowed to observe green fluorescing transconjugants appearing earlier and at higher ratio on donors during biofilm growth and maturation on PVC compared to PS [29]. The conjugal transfer on PVC was most probably favored by oxidative stress since ROS were more abundant in PVC and in the smaller particles, and, according to flow cytometry analysis, induced a dose-dependent membrane permeabilization in the recipient bacteria. An enrichment of DNA replication and repair pathways and QS signal transduction systems in smaller particles, was determined by functional prediction with Tax4Fun [30]. The PVC leachate obtained by rinsing MP particles promoted HGT while, in the case of PS, the solid particles rather than the leachate favored HGT. A Bliss independence model analysis [31] highlighted that the effect of PVC particles and leachate on HGT frequency was synergistic [29].

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Poly lactic acid (PLA) is the most widespread BDP whose production in the world is steadily increasing. It has a degradation time of decades and a high tendency to fragmentation with consequent debris accumulation in water and the release of large amounts of its highly toxic plasticizer dibutyl phthalate (DBP) that constitutes from 10% to 70% of its weight. PLA MPs and DBP after 12 h incubation increased of about two-fold the conjugation frequency between *E. coli* Stbl4 donor of a mobilizable plasmids encoding chloramphenicol and tetracycline resistance and *E. coli* JM109 recipient at 100 mg/L and 0.1–100 μg/L, respectively, and the combination PLA MPs 1 mg/L and DBP 1 μg/L slightly increased the conjugation frequency [32].

Transformation with the pAC plasmid encoding chloramphenicol resistance increased by 28% in presence of 10 to 100 mg/L PLA MPs and by 1.2 to 1.5-fold in presence of DBP reaching the highest level for the combination PLA MPs 1 mg/L and DBP 1 μg/L. ROS formation increased in the bacteria exposed to 1 to 100 μg/L DBP and was enhanced when also PLA MPs were present. Staining with 2′, 7′ - dichlorofluorescein diacetate (DCFH-DA) showed maximum membrane permeability in presence of 100 mg/L PLA, 100 μg/L DBP, and the combination PLA MPs 1 mg/L and DBP 1 μg/L. An increase in the total antioxidant capacity (TAOC), activation of the SOS response indicated by the upregulation of *umu*C and *lex*A, a 3-fold increase of EPS levels and adhesion, upregulation of the membrane permeability genes *omp*A and *omp*C and the mating genes *trf*Ap and *trb*Bp were observed in donor cells. In addition, DBP induced the upregulation of the DNA translocation, recombination, and replication genes *bhs*A, *yba*V, *nfs*B, and *rec*F [32].

Among the BDPs polyhydroxybutyrate (PHB) and PLA and the NBP PS NPs, the latter originated fewer NPs after exposure to light in water for 60 days. No toxicity of the NPs was observed on *E. coli* DH5α harboring the plasmid RP4 and *E. coli* HB101 used as donor and recipient, respectively, to analyze plasmid conjugation frequency. Exposure to PS NPs did not vary the plasmid transfer frequency possibly for their low concentration while for the BDPs an increase of the transfer frequency was observed mostly for PLA since PHB NPs were unstable in solution and aggregated. The increase intransfer frequency was of up to 28-fold for PLA NPs and up to about 13-fold for PHB NPs. An increase of about 2-fold of membrane permeability based on LDH release was observed upon exposure of the two strains to PLA NPs and the expression of proteins *omp*A and *omp*B was maximum with an increase of 6/7-fold. Both BDP NPs induced the upregulation of the global regulators *kor*A, *kor*B, and *trb*A with consequent increase of the *trf*Ap and *trb*Bp expression level [33].

PS spherical particles of about 1 μm size at 1 mg/L concentration, as possibly found in the water environment, increased the conjugative transfer frequency of plasmid RP4 from *E. coli* DH5α to *E. coli* HB101. However, its combination with 1 mg/L of the neurotoxic and endocrine disruptor plasticizer di (2-ethylhexyl) phthalate (DEHP) decreased the transfer frequency possibly for the increased hydrophobicity of the PS/DEHP combination [34].

The acquisition of phenotypic AMR occurred in *E. coli* ATCC 700926 let to attach for 24 h to 10 μm diameter PS spheres at concentrations ranging between 10,000 and 15,000/μL in LB broth and exposed to sublethal concentrations of ampicillin, ciprofloxacin, doxycycline, and streptomycin. The MIC for ciprofloxacin, doxycycline, and streptomycin increased by 100-fold after 10 days for the cells detached from the 500 μm PS MPs. Moreover, a 75 to 171-fold increase of multidrug resistance (MDR) was observed. After 10 days from the cessation of antibiotic exposure, the bacteria exposed also to MPs or to MPs alone retained or increased the level of AMR at a higher extent than those exposed only to the antibiotics, particularly those exposed to doxycycline and MPs. The minimum inhibitory concentration (MIC) for ciprofloxacin increased more than 3-fold after exposure to sublethal concentrations of this antibiotic and bigger PS particles and more than 2-fold for smaller particles reaching values higher than the clinical *E. coli* breakpoint in the presence of MPs only. Cells detached from MPs had an increased capacity to form biofilms in petri dishes, impaired motility and enhanced increase of ciprofloxacin MIC compared to planktonic cells [35].

Finally, PS MPs were found to increase the mutation rate, calculated as the ratio of bacteria acquiring resistance to rifampicin on total bacteria, in *E. coli* K12. This was maximum on particles functionalized with NH_2 to acquire a positive charge and with 0.1 μm diameter combined with

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norfloxacin at concentrations possibly found in estuary water. These combinations also promoted the highest conjugation frequency of plasmid RP4 between *E. coli* strains and the increase of ROS and membrane permeability [36].

4. Occurrence of ARGs and Bacterial Hosts on Plastic Fragments in Waterbodies

The experimental procedures adopted in the studies regarding the plastisphere formation, ARG selection and HGT in water environments are not described in depth in this review since they are detailed in a dedicated review that illustrates their advantages, disadvantages and evolution. These consisted in the analysis of the biofilm formed either after in situ deployment of MPs in cages in waterbodies or after MP suspension in laboratory scale microcosms containing waters from natural sites or suspensions that mimick their composition. Alternatively, the direct microbiological and biomolecular examination of plastic debris isolated from the aquatic environments and collected by pumping and filtration coupled with the identification of the plastic polymers and analysis of plastic degradation by Fourier transform infrared (FTIR) and Raman spectroscopy was also applied. SEM was adopted to observe plastic degradation, biofilm growth and EPS formation. The studies on the microbial community composition in the plastisphere were carried out either by isolation and molecular identification of the isolates by 16S rRNA gene sequencing or by whole DNA extraction from the plastic samples followed by high throughput sequencing (HTS) of 16S rRNA gene regions, e.g. V4, V6, V3/V4, V4/V5, and V4/V6, for prokaryotes or the 18S rRNA gene, the ribosomal internal transcribed spacers, ITS, and the 26S rRNA gene for eukaryotes [37]. This type of analysis is referred to as “metataxonomic” in this review.

ARGs and MDRGs were sought by quantitative Polymerase Chain Reaction (qPCR), high throughput qPCR (HT-qPCR) and/or metagenome sequencing that allowed to simultaneously identify the microbial composition of the plastisphere and the enriched cell functions. Bacterial abundance was generally determined as 16S rRNA gene copy number and the relative abundance of ARGs was expressed as copy number of ARGs on the 16S rRNA gene copy number. These parameters were compared with those in water or natural solid substrates such as sand, gravel or wood. Therefore, the definition “relative abundance” in this review is used with the above meaning, unless otherwise specified. Network correlation analysis was widely used to identify the most probable bacterial hosts of ARGs, ARG associations and ARG variation with environmental parameters [37].

A systematic review based on 69 plastisphere metagenome datasets from literature sources defined the correlation between the bacterial community abundance and ARGs in MPs from water environments by using the machine learning algorithms multiple linear regression (MLR), multilayer perceptron (MLP), gradient boosting decision tree (GBDT), and random forest (RF). Among the plastic polymers PVC recurred most often, followed by PE and polyhydroxialcanoate (PHA) and fibers were the MP shape most frequently recurring. The bacterial groups associated to MPs were, in order of abundance, Pseudomonadota, Actinomycetota and Bacteroidetes with genera diversity ranging from about nine hundred on PLA to more than two thousand on phenol formaldehyde (PF). ARGs reads per million decreased from more than one thousand to less than one hundred in the order PHA > PLA > PE > PS > PFP > PP > PF > PA and PET. More than 85% of the ARGs were MDRGs followed by ARGs for sulfonamides, tetracyclines and aminoglycosides in order of abundance. ARGs for macrolides, penicillin and rifampicin were also often reported. The enriched ARG types differed with the MP polymer and the BDPs presented the highest risk of ARG spread. All the four algorithms allowed to predict the presence of ARGs on the basis of the occurrence of bacterial genera and the MP type, particularly the MLR. A more accurate prediction of the ecological risks and the efficiency of ARG removal treatments will be achieved by taking into account the water environment, i.e. freshwater or seawater, MP aging, antibiotic occurrence and more numerous metagenomic datasets [38].

Salinity influences microbial diversity, abundance and ARG type in the plastisphere according to a study in which poly-butyleneadipate-co-terephthalate (PBAT) and PET MPs were submerged in freshwater and seawater in separate tanks for two weeks and then those in freshwater were

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transferred to seawater and viceversa for additional four weeks. The quinolone resistance gene *qnr*S and the sulfonamide resistance gene *sul*2 were dominant in the plastisphere in both water types but the tetracycline resistance gene *tet*A and *qnr*A were detected in seawater only after the transfer being most probably released from the plastisphere. Conversely, the macrolide resistance gene *mef*A became bound to MPs after the transfer being most probably already present in seawater. Rhizobiaceae, *Romboutsia* and *Clostridium_sensu_stricto*_13 decreased after MP transfer to seawater possibly inhibited by salinity. Network analysis showed that fewer bacterial genera in the PBAT plastisphere were positively correlated with the ARGs in seawater compared to freshwater while the opposite was observed for PET, particularly for *tet*A and the chloramphenicol resistance gene *cml*A1. When entering freshwater PBAT became colonized by genera positively correlated with *qnr*S, among which *Afipia* and the Rhizobiaceae. The genus *Bacillus* was positively correlated with *sul*2 and the beta-lactamase *bla*Q and the genera *Gemmobacter*, *Conexibacter* and *Lamia*, were positively correlated with *tet*A, *tet*C, *tet*X and the erythromycin esterase *ere*B. When MPs entered seawater, the MGEs *tnp*A04 and *tet*A, *qnr*S, and *bla*Q became strongly associated to the genus *Labrenzia* and the family Vicinamibacteraceae while *tnp*A05 co-occurred with *qnr*B in *Coxiella* spp., the Acidobacteriota *Sva0996_marine_group*, and genera *Croceibacter* and *Tumebacillus*, indicating HGT among different genera. The biodegradable PBAT enriched more ARGs than PET possibly for the release of carbon sources and rougher surfaces that favored bacterial adhesion [39].

The study mentioned above indicated that the microbial groups harboring ARGs in MPs in freshwater or seawater differ in composition so the following sections treat separately the studies regarding freshwaters, seawaters and waters with intermediate degrees of salinity.

4.1. ARGs in the Plastisphere in Freshwater

This section regards studies on the ARG occurrence in the plastisphere from rivers and lakes carried out in different countries.

One of the earliest investigations reported that the MPs collected from two urban rivers of Jiaxing City (China) in the Yangtze River Delta, constituted mainly by PE and PP, based on metataxonomic analysis, harbored a lower microbiota diversity than water with a significantly enrichment in Gammaproteobacteria, Bacilli, Anaerolineae and Firmicutes, and the genera *Deinococcus-Thermus*, and *Nitrospira*. The abundance of ARGs by qPCR was lower in MPs than in water except for some genes including *tet*M, *tet*S, and *tet*W. Co-occurrence analysis indicated Proteobacteria as ARG hosts in MPs and the integrase genes *intI*1 and *intI*2 enriched in MPs indicated a higher risk of ARG spread in the MP biofilm [40].

Metataxonomic analysis showed that at different time intervals during 30 days incubation in waters of an urban area the bacterial phylum mostly enriched in the plastisphere compared to water was Comamonadaceae followed by Planctomycetaceae. The enriched bacterial species were *M. abscessus*, *B. megaterium* and *P. putida*. The ARGs enriched in MPs based on HT-qPCR were the fluoroquinolone efflux pump *qep*A, *lnu*F, *aad*A7, *bla*$_{OXY-1}$ and *tet*G F. The ARG *erm*E was particularly enriched in MPs, and *flo*R, *cph*A and *aph*4-Ib were found only in MPs at some time intervals [41].

In the Dutch portion of the Rhine River sampled in winter and summer, the MP concentrations were higher in summer and those with 2-10 μm size were more abundant. PA and PVC prevailed but PE, PET, PP, PS, polyurethane (PU) and isoprene were also detected. Metataxonomic analysis showed that the genera *Flavobacterium*, *Simplicispira* and *Pseudomonas* were positively associated with PET, *Acinetobacter* with PP, *Hydrogenophaga* with PS, *Pseudarcicella* with isoprene, and *Synechococcus* with PU. The ARGs *sul*1 and *erm*B were specifically sought and showed a prevalence of about 94 and 99%, respectively in all samples [42].

PHB, low-density PE (LDPE) and high-density PE (HDPE) deployed for 11 days in an Arctic lake of the Svalbard islands (Norway) were colonized by Proteobacteria and the much less abundant phyla Cyanobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia. *Mycoplana* spp. predominated both on BDPs and NBPs followed by *Erythromicrobium*, *Zymomonas*, Comamonadaceae and *Rhodobacter*. Moreover, Sphingomonadaceae, *Pseudanabaena* and *Sphingomonas* were detected in

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NBPs and Moraxellaceae and *Polaromonas* in BDPs. The ARGs *sul*I and *erm*B were specifically sought by qPCR and detected in all polymers with higher abundance than in water and rock biofilm and both were particularly abundant in HDPE [43].

MPs, water, sediment, and wood particles were collected at eleven sites along the Ganjiang river (China) where the most abundant MPs were PE, PP, and polybutadiene (PBD) and in which metataxonomic analysis showed an enrichment in the genera *Flavobacterium*, *Rhodoferax*, cyanobacteria, an *Unindentified_bacterium*, and *Pseudomonas* spp., including the potentially pathogenic *P. protegens* and *P. stutzeri*. ARGs were not enriched in MPs, but their relative abundance and that of the *intI*1 gene were positively correlated hinting HGT. Genes *erm*F and *erm*B were found only in MPs and their most probable host was *Streptococcus mitis* [44].

Along the whole Beilun River at the China-Vietnam border characterized by areas with different degrees of urbanization the abundance of ARGs in MPs defined by HT-qPCR varied between multiples of 10^3 and 10^6 copies/g among 14 sites, were higher than in water and conferred resistance to beta-lactams, aminoglycosides, multiple drugs, MLS, tetracyclines, sulfonamides, vancomycin and chloramphenicol in order of abundance. IS6100, IS26, and *tnp*A-6 were the MGE indicators most frequently detected. ARG and MGE abundance and number increased from rural to urban regions, particularly aminoglycoside, MDR and tetracycline ARGs, and decreased slightly along the estuary. Thirty ARGs among which *tet*A, *tet*B, *sul*1 and *qac*H predominated and were detected at all sites. In peri-urban and urban areas some genes such as *aac*(6′)-Ib, *bla*$_{VEB}$, *qnr*B4, *erm*B, *pbr*T, *tet*E, the integron encoded trimethoprim resistance dihydrofolate reductase *dfr*A1, *van*G and *mex*B were newly found. In the rural regions ARGs were more abundant in PE, in the peri-urban regions in PE and PF and in the urban regions in PP. This was explained with the larger SSA of PE and PP. Aminoglycoside ARGs were enriched in MPs compared to water and mostly on PE while the most abundant ARGs in PP were *tet*B and *tet*G. Network correlation analysis showed that IS6100 co-occurred with 20 ARGs in peri-urban and urban areas and that the genera *Vibrio*, *Flavobacterium*, and *Chryseobacterium* carried at least five ARGs. The latter genus occurs in clinical settings. MDR bacteria such as the genera *Muriicola*, *Robiginitalea*, and *Woeseia* potential host of up to 39 ARGs occurred in urban areas while bacteria in the rural area showed at most of 16 co-occurring ARGs. Socioeconomic factors population density, presence of hospitals and levels of domestic sewage were positively correlated with ARG occurrence. In particular the population density was positively correlated with aminoglycoside and sulfonamide ARGs. These factors were correlated with MGEs as well and explained 88% of the ARG occurrence variance. In the rural areas ARGs were better correlated with the bacterial composition compared to the peri-urban and urban regions. The projection pursuit regression (PPR) model allowed to highlight that all MP types except pentafluorophenyl acrylate (PFP) showed an increased ARG dissemination risk with urbanization that followed the order PP > PE > PS > PF > PFP [45].

In PE and PS MPs deployed for 30 days in two sites of the Houxi River watershed (China) dominant taxa in the plastisphere were Proteobacteria, Bacteroidetes, and Cyanobacteria. Oxyphotobacteria were enriched in MPs in a natural reserve area while α-Proteobacteria were enriched in the bay area. The number of ARGs in the plastisphere was significantly lower than in water and even more in the natural reserve area but a positive correlation was found between the relative abundance of ARGs and MGEs. The bay MP samples carried a higher abundance of potential pathogens than water among which *K. pneumoniae* and *Enterobacter cloacae* associated with at least two ARGs [46].

In the Huangpu River (China) sampled at ten sites PET fibers were the main MP component followed by PA, polymethyl methacrylate (PMMA), PE and PP. Metagenome sequencing showed that the relative abundance of ARGs for tetracycline and chloramphenicol was higher and ARGs for rifamycin and vancomycin were selectively enriched in MPs. ARG profiles were more diverse in MPs than in water with a total of 313 subtypes and subtypes of the lyncosamide resistance gene *lnu*A, a not specified tetracycline resistance gene, the integron-encoded ribosyltransferase *arr* conferring rifampicin resistance, the efflux pump component *mex*I, *bla*$_{LRA-12}$, and the efflux pump gene *rosA* were selectively enriched. MGEs associated to MPs were less diverse than in water but the plasmid genes

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*Rep*13, *Rep*21, *Rep*7, *tnp*AB, linked to 13 subtypes of ARGs, and *ISRj*1 were more abundant. Moreover, *rep*21 and *lnu*A, *Rep*7 and the tetracycline resistance gene showed strong positive correlations indicating HGT potential for these ARGs. Correlation network analysis showed that the Proteobacteria genus *Afipia* was highly correlated with 28 ARGs, including the aminoglycoside acetyltransferase *aac*(2′)-*I*, *arr*, the chloramphenicol acetyltransferase *cat*, *mex*I, *bla*$_{\text{TEM-1}}$, and *tet*V [47].

PE, PVC and PET MPs were incubated in bottles containing filtered river water for one month in presence of antibiotic-resistant bacteria (ARB) carrying the ARGs *tetA*, *tetC*, *tetO*, *sul1* and *intI*1 or in presence of extracellular ARGs *tetA* and *bla*$_{\text{TEM}}$ in plasmids. The ARB studied had been selected by incubating the river water samples in presence of 0.1 mg/L tetracycline for five days and isolating the bacteria grown on LB medium supplemented with antibiotics. After five days all the intracellular ARGs were detected on the MPs with higher relative abundance in PET and PVC and with *sul1*, *tetA*, and *intI1* at three orders of magnitude higher than *tetC* and *tetO*. Extracellular *tet*A decreased in MPs in the first 15 days and increased later while *bla*$_{\text{TEM}}$, already at initial levels of 9 Log copy number/g, further increased. The bacterial genera identified in MPs by metataxonomy and positively correlated with the intracellular *tet*A, *tet*C, *sul*1 were *Pseudomonas*, *Solobacterium*, *Achromobacter*, *Aeromonas*, *Beggiatoa*, *Propionivibrio*, and *Paludibacter*, with the latter three genera also positively correlated with *tetO* while *intI*1 was positively correlated with *Tolumonas*, *Pseudorhodobacter*, and *Rhodoferax* [48].

PLA and PVC MP biofilms incubated for 14 days in bioreactors containing water from the Haihe River (China) were analyzed by metataxonomy, shotgun metagenomic and metatranscriptomic. The PLA biofilm was the most diverse with enriched Planctomycetes, Spirochaetes, Gemmatimonadetes, *Deinococcus-Thermus*, Tenericutes, Fibrobacteres, and Cyanobacteria. In the PVC biofilm *Deinococcus-Thermus*, Tenericutes, Epsilonbacteraeota, Actinobacteria, Cyanobacteria, and Bacteroidetes were enriched. Metagenomic analysis identified the macrolide resistance gene *macB* and MDRGs, in particular *ceoB*, part of an efflux system conferring resistance to chloramphenicol, trimethoprim, and ciprofloxacin, as highly abundant in the plastisphere. The diversity of ARGs was the highest on PLA with 173 ARGs detected, among which 75 encoding resistance to 18 antibiotics and predominantly MDRGs were transcribed and showed a higher expression level in MP biofilms. Twenty-nine ARGs and 13 ARG transcripts were detected only in the plastisphere. Alignment with the plasmid protein database indicated that some ARGs expressed at a higher extent in the plastisphere than in water were plasmid encoded, thus with HGT potential. Correlation networks showed that ARGs were distributed mainly in the phylum Proteobacteria, followed by Firmicutes, Bacteroidetes, Desulfobacteraeota, Actinobacteria, and Desulfuromonadaeota. Among the identified bacterial hosts *E. cloacae*, found only in PLA, expressed the *tet*G gene [49].

PET and PBAT incubated in waters collected in the West Lake, a canal and the Qiantang River (China) for two weeks showed an increased relative abundance of the ARGs initially present in water more remarkable in PBAT, particularly the *tet* genes. When the MPs were transferred to another water sample some ARGs such as *tet*C, *tet*G, *sul*1 and *sul*2, were released in the receiving water. Conversely, *cml*A1 present in water increased in the PBAT MPs and was no more detected in water. Positive correlations of ARGs with *intI*1, particularly in PET, and with *tnp*A05 were found [50].

The pathogenic bacterium *Morganella morganii* that can cause severe infection in different organs, was isolated from Artificial Plastic Substrates (APSs) submerged in the Bracciano lake (Italy) for one month. The isolates carried *intI*1 and the ARGs *tet*C, *sul*1, *sul*3, the chloramphenicol resistance genes *cml*A1 and *cmx*A and the extended-spectrum beta-lactamases (ESBL) *bla*$_{\text{CTX-M-01}}$ and *bla*$_{\text{CTX-M-02}}$ [51].

PP and PET MPs after 30 days of incubation in microcosm experiments with river water showed a more abundant biomass compared with gravel rock reaching numbers of the order of 10^5 CFU/g. The gene *tet*B was present in PP as also the ARB genera *Pedobacter* and *Pseudomonas* [52].

The biofilms in PVC, PE, polycaprolactone (PCL) and PLA MPs deployed at ten sites with different density of urbanization in the Houxi River (China) and analyzed by metagenomic sequencing showed an abundance of MDRGs, and ARGs to macrolide-lincosamide-streptogramin (MLS), sulfonamide and polymyxin, and MGEs increasing with the bacterial population density. The most prevalent ARGs in urbanized sites were the bacitracin resistance gene *bac*A, the polymyxin B

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resistance gene *ugd* (*pmr*E), *sul1*, *sul2*, the multidrug resistance transporter *msbA* and the efflux pump component *acr*B. The most prevalent MGEs were *tnpA*, *tnpA1*, *tnpA2*, *tnpA3*, *tnpA5* and *intI*1. Aminoglycoside resistance genes significantly increased on PCL and PVC while quinolone resistance genes increased in PLA and PE. The abundance of MGEs in MPs followed the order PVC < PCL < PE < PLA. Forty-eight metagenome assembled genomes (MAGs) assigned to Burkholderiales, Pseudomonadales, Enterobacterales and to the genera *Pseudomonas* and *Aeromonas* presented the highest ARG risk. The human fecal marker crAssphage was positively correlated with ARGs and *intI*1, indicating that human fecal pollution caused the ARG increase [53].

Metataxonomic analysis of the plastisphere of PET, PP and Mater-Bi (Mater-Bi, Novamont, Italy) submerged in the Lake Maggiore (Italy) for 43 days showed a distinct composition of the microbiota and a higher abundance of potential pathogens, including the genera *Streptococcus*, *Rickettsia*, *Shewanella* and *Sphingomonas*, than water. The microbial diversity was greater in the plastic materials compared to cellulose but lower than in wood. ARGs were more abundant on PET and PP, in particular *tet*A, while the *intI1* gene was more abundant on Mater-Bi and PET [54].

MPs collected from the surface water of the Poyang Lake, the largest freshwater lake in China and National Natural Reserve, were constituted mainly by PE and PP. Shotgun metagenomics showed that these harbored a less diverse microbiota than the surrounding water dominated by Proteobacteria followed by Actinobacteria. *P. fluorescens* was the most abundant pathogen and *A. junii*, *A. caviae*, *A. sobria*, *Brevundimonas diminuta*, *K. pneumoniae* and *Rahnella aquatilis* were also detected. The relative abundance of ARGs for bacitracin, beta-lactams, polymyxin, fosfomycin, quinolones and sulfonamides, and their subtype number was significantly higher on MPs with 184 unique subtypes in PE and 46 in PP. The MDRG *mexC*, *bla*$_{\text{LRA-13}}$ and *fosA7*, were detected only in MPs. The genes *bacA*, *bla*$_{\text{THIN-B}}$, and the MDRGs *mdt*B, *mex*E-*mex*F-*opr*N were among those mostly enriched in PP while *mdt*B was enriched in PE. Network correlation analysis showed that *A. veronii* was associated with the beta-lactamases *bla*$_{\text{OXA-12}}$, *cph*A6, *cph*A8, and *cph*A3 and *Arthrobacter* spp. with many ARGs, among which the MDRG *mds*B, the novobiocin exporter *nov*A, the rifampin phosphotransferase *rph*A, and *emr*K. Moreover, three novel beta-lactamases were identified with the fARGene software [55]. These showed extended spectrum beta-lactamase (ESBL) and carbapenemase activities after cloning and expression in *E. coli* BL21 [56]

Metagenome sequence analysis, showed that PBAT, polybutylene succinate (PBS), PHA, PLA, PP, PE, PVC, and PS MPs deployed in situ in four lakes in Wuhan (China) for two months contained a more diverse microbiota and a larger number of opportunistic pathogens than water. Proteobacteria predominated on all the polymers, *Firmicutes*, *Nitrospiria*, and *Verrucomicrobia* were enriched on PE, PP, PS, PVC, and PLA while Proteobacteria and *Candidatus* Parcubacteria were enriched on PHA, PBS, and PBAT. Among pathogens, *Cystobacter fuscus* was the most abundant in all MPs, *Ralstonia solanacearum*, *Burkholderia cenocepacia*, and *B. glumae* were enriched on the PHA, PBS, and PBAT and *Coxiella burnetii*, *Legionella pneumophila*, and *Xylella fastidiosa* on the other plastics. A null-model-based stochasticity index highlighted that the ARG assembly was dominated by stochastic processes in PP and by deterministic processes in the other MPs. MGEs, mainly transposases, were enriched and differently distributed among the plastic polymers. Network analysis showed a higher number of potential ARG hosts in BDPs among which *Riemerella anatipestifer*, an avian pathogen, was associated with the MGEs *IS621*, *ISBaba6*, *ISBf10*, *ist*A, and *tnp*A27 and to the ARGs *nov*A, the macrolide exporter *mac*B, *mds*B, the multidrug efflux pump *sav1866*, the tiamulin efflux pump *tae*A, the polymyxin resistance gene *arn*C, and the quinolone resistance gene *mfd*. *Vibrio campbellii* was associated with the MGEs *ISBaba6* and *tnp*A and with the ARGs *nov*A, *bla*$_{\text{CRP}}$, *tae*A, the polymyxin resistance gene *pmr*E and *tet*4 while *V. cholerae* was associated with the amoxicillin resistance gene *pbp1a*, the fosfomicin resistance gene *mur*A, the efflux pump *efr*A, the macrolide efflux pump *mtr*A, and *tet*PB and the MGE *ist*A15 [57].

Twenty-two bacterial isolates obtained from plastic substrates present in lake water and identified by 16S rRNA gene sequencing as *Lysinibacillus* spp., *Exiguobacterium acetylicum*, *Klebsiella pneumoniae*, *K. oxytoca* and *K. michiganensis* were submerged in a lake for 30 days in presence of MPs

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and adhered to the particles at rates decreasing in the order PS, styrene acrylonitrile resin (SAN), and, equally, PP and PET. *Klebsiella* spp. was re-isolated from PS and SAN only. After re-isolation, all five bacterial groups shared the gene *bla*$_{TEM}$, *Lysinibacillus* spp. and *Klebsiella* spp. shared *bla*$_{SHV}$, *Lysinibacillus* spp., *E. acetylicum* and *K. michiganensis* MDRG *ade*A, *Lysinibacillus* spp., *E. acetylicum* and *K. pneumoniae tet*A, *Lysinibacillus* spp., *K. pneumoniae* and *K. oxytoca* the efflux pump component *acr*B, *Lysinibacillus* spp., *K. pneumoniae* and *K. michiganensis sul*1, *Lysinibacillus* spp. and *K. pneumoniae bla*$_{CTX-M}$, *Lysinibacillus* spp. and *E. acetylicum* the beta-lactam resistance gene *mec*A, *Lysinibacillus* spp. and *K. michiganensis tet*W and the efflux pump *acr*F, *E. acetylicum* and *K. pneumoniae cmx*A and *K. pneumoniae* and *K. oxytoca sul*2. In addition, *K. oxytoca* harbored *bla*$_{CTX}$ and the MDRG *acr*R, and *K. michiganensis* harbored *tet*B. The genes *intI*1 and *intI*1V were detected in almost all isolates. These results indicated that HGT events occurred for most of the ARGs detected and involved all the bacteria studied [58].

MPs distribute at different depths in waters according to their density, size and shape. Therefore, it was investigated how ARGs distribute vertically in PET and PLA MPs deployed in the Qinhuai river (China) during 60 days. At higher depth, biofilm formed more rapidly on PLA but the biofilm biomass was lower compared to lower depths as a consequence of lower temperature and oxygen availability. However, the relative abundance of ARGs, including *qnr*S, *bla*$_{NDM-1}$, which encodes the superbug New-Delhi metallo-beta-lactamase-1 for resistance to most beta-lactams and carbapenems, *flo*R, *sul*1, *qnr*A, *tet*G, and the colistin resistance gene *mcr*1 encoding an enzyme that modifies the affinity of lipid A for this antibiotic, increased more remarkably in MPs than in water, and particularly in PLA. Metataxonomy showed that the genera *Kouleothrix* and *Nitrospira* were enriched in PLA and *Hydrogenophaga* and *Flavobacterium* were enriched in PET. Correlation analysis linked *Hydrogenophaga, Nitrospira, Methyloversatilis*, and *Ellin6067* to ARGs in PLA, and *Dinghuibacter, Ahniella, Dechloromonas*, and *Acinetobacter* to ARGs in PET. The potential of HGT, indicated by the abundance of *intI*1 and *tnp*A05, increased with depth. The *intI*1 gene prevailed and was more abundant in PET [59].

A subsequent study carried out in the same river found that proximity to the sediment led to an increase of ARG abundance and HGT probability more in PET than in PLA MPs after incubation in cages in deep water for 30 days and further incubation of a group of cages at the sediment-water interface for additional 30 days. The abundance of ARGs determined by HT-qPCR in deep water was nearly double in PLA compared to PET while at the sediment-water interface the ARGs abundance in PLA decreased by more than 50%, and in PET it increased more than 3-fold. The concentration of nitric and ammonia nitrogen, and phosphates were positively correlated with most ARGs and these increased at the sediment-water interface showing that the availability of nutrients favored ARG increase in PET. Network correlation analysis indicated that the genera *Sphingomonas*, *Nitrospira*, *Nitrosomonas*, *Flavobacterium*, and *Kouleothrix* were positively correlated with most ARGs. The total abundance of MGEs in PLA slightly decreased while it increased of about 1.5-fold in PET, indicating a higher probability of HGT in this polymer. The MGEs *tnp*A1, *tnp*Acp2, *tni*A, IS91, and *intI*1 were positively correlated with ARGs, including the aminoglycoside nucleotidyltransferase *aad*A, *sul*1, *sul*2, *efr*B, and *erm*F [60].

Processes favoring HGT were upregulated in PET and downregulated in PLA. These included QS, as indicated by an increased amount of AHLs autoinducers and the upregulation of *arg*AB, *arg*D, *arg*J, *lsr*G, *rpf*C, and *ahl*D, membrane permeability, as indicated by a higher release of the intracellular enzyme lactate-dehydrogenase (LDH) and upregulation of *sec*A, *gsp*D, *yid*C, *sec*Y, *srp*54, *fts*Y, *tol*C, and *gsp*E, ROS production, as indicated by the upregulation of *kat*G, *kat*E, *gpx*, *alk*B, *sox*X, *SOD*1, and *SOD*2, stress response as indicated by the upregulation of DNA integration and repair genes *rec*A, *uvr*A, *uvr*B, *dna*E, and *pol*A, polysaccharide synthesis, as indicated by the upregulation of *lpx*A, *lpx*B, *lpa*D, and *lpx*H, and protein export, as indicated by the upregulation of *tat*A, *tat*B, and *tat*C. Moreover 11 components of the T4SS system, in particular *vir*B10 encoding an inner and outer membrane translocase and *vir*D4 encoding a receptor for substrate conjugation including ARGs, were upregulated in PET [60].

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In MPs sampled at multiple sites from the middle to the final part of the Red River (Vietnam) the abundance of the biofilm biomass was not correlated with the plastic polymer type except for PET and polytridecanolactone (PTDL). Bacteria were isolated on MacConkey agar supplemented with 1 µg/mL cefotaxime from almost all sampling sites and their highest concentration was of the order 10^5 CFU/mL on MPs from industrial sites and more abundant in freshwater. The presence of Chlorophyll-a originating from primary production was positively correlated with ARB abundance on MPs. Among 207 isolates, 99% were identified by 16S rRNA gene sequencing as *Aeromonas* spp. including *A. veronii* and *A. caviae* while *E. coli* was isolated only from one site. ESBL genes were detected in 23 isolates by PCR among which *bla*$_{SHV}$ from one site, *bla*$_{TEM}$ and *bla*$_{SHV}$ from another site and these genes plus *bla*$_{CTX-M}$ from a site receiving hospital effluents [61].

PE, PP, PS, PLA, and PHA MPs were incubated for 35 days in the Central Lake (China) and EPS formation and biomass increased during four weeks to up to multiples of 10^7 cells/g. The order of biomass abundance was PHA > PLA > grit used as NS > PP and PS. Shotgun metagenomic analysis showed that Plantomycetes were present only in MPs, Alphaproteobacteria were enriched in PE, PP and PS and Betaproteobacteria were enriched in PHA and PLA. The pathogens *P. aeruginosa*, *P. fluorescens*, *S. enterica*, *Mycobacteroides abscessus*, *Burkholderia pseudomallei*, and *K. pneumoniae* showed a lower relative abundance on NBPs than in water and BDPs. *Shigella flexneri* and *E. coli* were enriched in PLA and *P. aeruginosa*, a WHO priority 1 pathogen [62], in PHA. The relative abundance of ARGs was slightly higher on MPs than in water in the order PHA > PLA > PS > PE > PP. The relative abundance of MGEs was higher on BDPs with ICE *SXT/R391* enriched in PHA and *intI*1 in both BDPs. Co-occurrence network analysis showed that 74 ARGs and 29 MGEs shared positive correlations. Proteobacteria, mainly *P. fluorescens*, *Xanthomonas campestris* and *X. citri*, were the main ARG and MGE hosts and were more abundant in MPs, particularly in BDPs. ARGs classified as rank I risk, based on co-occurrence with MGEs and occurrence in pathogens [63], namely *bacA*, *mecA*, *qacA*, *tolC*, *dfrA1*, the macrolide phosphotransferases *mphA*, and *mphB*, were more abundant in PHA [64].

In six laxes sampled in the Jiuzhaigou karst plateau (China) in 2023 fibrous MPs prevailed and were identified as PE, PET, PP and PS. Metagenome sequencing showed the predominance of Proteobacteria, Bacteroidota, and Actinobacteria in the plastisphere and higher abundance of Actinobacteria and Chloroflexi in MPs from the sediment. ARGs in the plastisphere were shared with water and sediment but showed a higher alpha-diversity in the MPs. The most abundant ARG was the MDR gene *evg*S [65].

Urban water bodies, namely an urban park lake, an urban river trait, and the urban-rural lake in Chengdu (China) were contaminated by PP, PE and PS and nylon MPs, respectively. In all three sites metataxonomy showed the presence of Proteobacteria as the most abundant phylum, Chloroflexi, Firmicutes, Actinobacteriota and Cyanobacteriota and the genera *Acinetobacter*, *Rhizobium* and *Exiguobacterium*. *Pseudomonas* spp. and *Acinetobacter* spp. were particularly enriched in the river. Metagenome sequencing revealed more than 100 ARGs at each site with 72 in common. The most abundant ARG at all sites was *bac*A, followed *sul1*, *sul2*, *ceoB*, the MDR efflux pump component *smeE*, *acrB*, *tet39* and *tetC*. The the aminoglycoside O-nucleotidyltransferases genes *ant(2″)-Ia*, *ant(3″)-Ia* were detected in the river. Based on the presence of MGEs, mainly transposases and recombinases, the risk of HGT was similar in the three environments. Integrases were detected only in the river. Among the bacterial groups carrying MGEs *Acinetobacter* spp. prevailed in the river and *Rhodopseudomonas* spp. in the rural lake. Aminoglycosides, bacitracin and tetracyclines ARGs were mostly chromosomal, while sulfonamides and rifampicin ARGs were mainly plasmid encoded. Network correlation analysis showed positive correlations of pathogenic Gammaproteobacteria, Pseudomonadota, Acidobacteriota and Actinomycetota with *bac*A and *sul*1 in the urban lake, of pathogenic Deltaproteobacteria and Desulfobacterales and *sul*1, *sul*2, *acr*B, and *ant*(2″)-Ia in the river A and pathogenic Gammaproteobacteria with *sul*1, *sul*2, *sme*E in the rural lake. Moreover, pathogenic Gammaproteobacteria and Pseudomonadota in the urban lake were positively correlated with *ist*A, *ist*B, *tnp*A, and *tnp*A-1, pathogenic Pseudomonadota and Hyphomicrobiales in the river were

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positively correlated with *ist*A3, *ist*B1, IS91, and *tnp*A and pathogenic Pseudomonadota in the rural lake were positively correlated with *tni*B, *int*I1 and *qac*Edelta MGEs [66].

Metagenome sequencing of the plastisphere in additive-free BDPs polycaprolactone (PCL) and PHB MPs and the NBPs PP and PS and of the biofilm on wood used as NS control deployed in a river in Shenzhen (China) for four weeks revealed that the BDP plastisphere pose a higher risk score (risk I-III) than the microbiota in the other sample types for its highest ARG dissemination risk. In the study the CompRanking workflow was developed to assess the contribution of the plastisphere resistome to environmental and health risks. This resource is freely available at https://github.com/GaoyangLuo/CompRanking (accessed on 29 September 2025). The risk scale used considered as risk III contigs comprising ARGs, as risk II contigs with co-located ARGs and MGEs, and as risk I contigs with co-located ARGs and MGEs from pathogens. The risks deriving for each contig type for the MPs were expressed as ratio contig type number/total contig number. It was observed that the MDRGs *omp*R, *opr*M, *mex*E, *gol*S, *emr*B, *mtr*D, *ceo*B, *ade*F, and ARGs polymyxin resistance efflux pump *ros*B, the beta-lactamase IND-6, *bac*A, the aminoglycoside ARG *kdp*E and the kasugamycin ARG *ksg*A were enriched in BDPs. Among these, *bac*A was particularly abundant and only *ceo*B and *ros*B were also moderately enriched in NBPs together to *oqx*B compared to water and wood. The defined AMR risk was BDPs > Water > Wood > NBPs. All particles including wood showed a higher prevalence of ARGs associated to MGEs than water thus a higher HGT risk. Moreover, in the BDPs ARGs were mainly distributed among phages while in the NBPs these were mainly linked to plasmids indicating a different contribution of transduction and conjugation to HGT in the two plastispheres and generalized transduction by phages could lead to a more efficient ARG spread in BDPs than conjugation in NBPs. The SOS-related genes *rec*A, *lex*A, *uvr*A and ROS-related genes *sod*B, *aph*C and *sox*R, significantly correlated with the AMR Risk Score, were more abundant in BDPs than in other sample types and a higher percentage of MAGs from BDPs carried virulence factors. Some identified genomes, such as *SYFN01*, *Xanthobacter* and *Allorhizobium* contained MGEs close to ARGs being most probably involved in ARG dissemination [67].

4.1.2. The Effect of WWTP Effluents on the Plastisphere ARG Content

Hotspots for the association of ARGs to MPs are WWTPs effluents that can cause the pollution of waterbodies if MP removal is not complete [68]. WWTP effluents are the main source of MPs in rivers and even those with a 99.9% removal efficiency release numbers of MP particles of the order of 10^5 per day while with a 98% removal efficiency release multiples of 10^9 MP particles per day [69]. Indeed, MPs can persist after the wastewater treatment process because of their resistance to sedimentation [70]. Moreover, MPs decrease the efficiency of ARG removal in WWTPs which are a hotspot of drug-resistant bacteria, ARG concentration and selection [69]. In addition, it was reported that UV disinfection enhanced the conjugation frequency between the donor *E. coli* DH5α harboring plasmid RP4 and streptomycin-resistant enterotoxigenic (ETEC) *E. coli* in a solution simulating wastewater in presence of 0.02 g/L and 0.1 g/L of PS and PLA MPs, mostly for PS at the lower MP concentration. The *E. coli* ARGs *tet*W and *tet*C and *int*I1 disappeared after UV treatment in absence of MPs while they increased in presence of MPs that exerted protection effects on the bacterial host. Protection was determined by the light shielding effect of the MPs that was more intense for PLA. The conjugative transfer was favored by the adhesion of bacteria to MPs and mostly on PLA, that released a higher amount of carbon nutrients, and was enhanced by the upregulation of EPS export genes and of the mating pair formation and replication genes *tra*F, *tra*G and *trf*Ap. Moreover, the generation of NP fragments upon irradiation increased oxidative stress and membrane permeability in bacteria mostly for PS that released maller NPs [71].

A highly infectious potential of the biofilms formed in PE MPs submerged for 14 days in a WWTP effluent was demonstrated by using *Galeria meillonella* larvae in which the detached biofilm was injected. Metataxonomic analysis showed that the human pathogens *Serratia marcescens*, *K. pneumoniae*, *A. hydrophila, Leclercia adecarboxylata* and *Enterobacter sichuanensis* which were detected at 0.5% relative abundance before injection, became dominant in the insect 24 h post-injection with

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Serratia spp. and *Klebsiella* spp. at a relative abundance of 73% and 76% with a survival reduction of 30% compared to the biofilm formed upstream from the WWTP discharge [72].

In a chemostat experiment simulating the introduction of treated wastewaters into waters from the Maggiore Lake (Italy) PS MPs favored the persistence of bacteria deriving from the treated wastewater. Automated ribosomal intergenic spacer analysis (ARISA) showed a bacterial diversity higher on the MPs than in water where diversity decreased with the increase of MP concentration. Quantification by qPCR of the 16S rRNA gene copies and the integrase gene *intI*1 showed that the relative abundance of MGEs in MPs increased with MP concentration in the plastishere but not in water [73].

Tetracycline, that was frequently detected in WWTP effluents, was tested for degradation in the plastisphere of PVC and PLA MPs incubated for 28 days in river water. PLA and PVC adsorbed more tetracycline than quartzite used as control and MP biofilms showed a simpler microbial composition than those formed on quartzite particles. The ARGs *tet*A, *tet*C, *tet*M, and *tet*X and the integrase gene *intI*1, detected by qPCR, were more abundant in MP biofilms as also the probable bacterial carriers of those genes identified by metataxonomy as *Pseudomonas* spp., Flavobacteriaceae, and actinobacteria [74].

Principal coordinate analysis (PCoA) of metagenomic data from LDPE and waste LDPE (W-LDPE) incubated for one week in the River Sowe (United Kingdom) showed that the most abundant bacterial genus in all biofilms was *Sphaerotilus*, an iron catcher and EPS producer that favors the adhesion of other microorganisms. *Acinetobacter* spp and *Aeromonas* spp. were abundant in all the biofilms while *Pseudomonas* spp., including *P. aeruginosa*, were significantly more abundant on W-LDPE which also showed a higher relative abundance of ARGs. HT-qPCR targeted on 48 ARGs allowed to observe that *qep*A was mostly enriched in wood and PE and *sul*1 in PE and PP incubated ex situ in a river water and sediment microcosm in presence of azithromycin, ciprofloxacin and sulphametazole. These had concentrations three orders of magnitude lower than the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (www.eucast.org, accessed on 25 July 2025) simulating concentrations in WWTP effluent [75].

Vancomycin-resistant *A. hydrophila* and *P. aeruginosa* and sulfamethazole-resistant *Bacillus cereus* isolated from the effluent of a WWTP in Madrid (Spain) were incubated in presence of MPs in synthetic WWTP effluent with different total organic carbon (TOC) concentrations. On day 7, SEM showed biofilm maturation and detachment indicating ARB dispersion. Addition of 20 µg/L vancomycin, ciprofloxacin, sulfamethoxazole, and ampicillin antibiotics led to a reduction of ARB in MPs but *sul1*, the vancomycin resistance gene *van*A and *intI1* were detected only in the MP biofilm and increased at TOC values higher than 15 mg/L. The addition of 80 µg/L antibiotics caused the disappearance of *intI*1, the decrease of *sul*1 and the increase of *van*A. In a real WWTP filtered effluent a greater survival of the bacteria naturally present and a concentration of ARGs and *intI*1 higher than in water were observed in PS MPs biofilm. SEM showed the formation of a biofilm after 30 days also for MPs incubated in tap water indicating that MPs can favor ARGs and *intI*1 selection even in household water [76].

In PE, PET, PP MPs, deployed singly or in mixture upstream and downstream from a WWTP discharge for 28 days in the Mondego River (Portugal), ciprofloxacin-resistant bacteria were more abundant than in sand, particularly in the downstream site, and on PP. These belonged mainly to the genus *Aeromonas*, and those resistant to cefotaxime to the genus *Pseudomonas*. Fifty-four Enterobacteriaceae isolates resistant to antibiotics and with unique BOX-PCR profiles derived from the MP mix were identified by 16S rRNA gene sequencing, matrix-assisted laser desorption ionization (MALDI) mass spectrometry and phenoptypic tests as *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *K. pneumoniae* and *Shigella* species. All except one were strong biofilm formers and most were resistant to ciprofloxacin, levofloxacin, piperacillin, ticarcillin, ticarcillin/clavulanic acid, gentamicin, sulfamethoxazole/trimethoprim, tetracycline, aztreonam and cefepime. These harbored the ARGs *aacA4-cr*, *qnrS*, *qnrB*, *qnrVC*, and *bla*$_{\text{CTX-M}}$ which was the most prevalent gene with the subtypes *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-32}}$ and *bla*$_{\text{CTX-M-55}}$ flanked upstream by a IS*Ecp1* insertion sequence and downstream by

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*orf*477. Two *Enterobacter* spp., 5 *E. coli*, and 3 *K. pneumoniae* strains were able to transfer by conjugation the gene *bla*$_{CTX-M}$ to *E. coli* CV601 which acquired resistance to piperacillin, ticarcillin, aztreonam, ceftazidime and cefotaxime [77].

By using the same experimental set-up and metataxonomy, it was shown that PP harbored a more complex microbiota than water both upstream and downstream from the WWTP with a clear PCoA separation between upstream and downstream samples, between PP and PET and between the MP mixture and each polymer. Comamonadaceae and Weeksellaceae increased in downstream MP samples that harbored the fecal bacterial genera *Bifidobacterium*, *Enterobacter*, and *Escherichia-Shigella*. The pathogenic genera *Acinetobacter*, *Aeromonas*, *Arcobacter*, *Clostridium*, *Flavobacterium*, *Legionella*, *Mycobacterium*, and *Pseudomonas* were associated with all MPs and *Bacillus* spp. and *Treponema* spp. with at least one but not with water. The genera *Clostridium*, *Mycobacterium* and *Flavobacterium* were significantly enriched in PET, and *Arcobacter*, *Pseudomonas*, *Citrobacter* and *Clostridium* in the MP mixture. The genes *aad*A2, *bla*$_{GES}$, the quaternary ammonium resistance genes *qac*EΔ1 and *qac*H and the transposase gene *tnp*A, were only detected in MPs and the total abundance of beta-lactam and sulfonamide ARGs and integrases were higher in the plastisphere than in water. The genes *bla*$_{CTX-M-5}$, *bla*$_{VEB}$, *bla*$_{VIM}$, *sul*2, *ere*A, the macrolide resistance system *mat*A/*mel*, and *intI*1 were enriched in the MP mixture and in PET which showed the highest abundance of MDRGs at both sites. The genes *aad*A2, *bla*$_{IMP}$, *bla*$_{OXA-10}$, *bla*$_{SHV}$, *sul*1 were enriched only in the MP mixture. ARGs for sulfonamides and MGEs were more abundant on PP [78].

LDPE, PET, PS, PVC items and the control substrates glass and rock were deployed for one year in a site surrounded by natural areas (site 1) and one downstream of a WWTP discharge (site 2) in the Henares River (Spain). Seven antibiotics were detected at a total concentration of about 300 ng/L at site 1 and ten at almost one hundred times higher concentration at site 2. SEM showed that the biofilm started to form during the first month, with presence of EPS after three months and appeared multilayered after six and twelve months. Metataxonomic analysis indicated a higher diversity of bacterial populations on plastics at site 2. Diversity increased on all polymers during time at site 1 except for PVC, possibly for the release of toxic substances by this material, while at site 2 bacterial diversity decreased, except for LDPE. Proteobacteria followed by Bacteroidetes and Cyanobacteria dominated in all particles. Among the ARGs specifically sought *ermF* was present at the highest concentrations on plastics after 6 months and *qnrSrtF11A* was more abundant on plastics and glass after three months [79].

Metataxonomy showed a higher microbial diversity than in water for HDPE, LDPE, PP, and PS MPs incubated for 10 weeks in treated wastewater microcosm. In the MP biofilms *Pseudomonas* spp., *Hyphomicrobium* spp., *Mycobacterium* spp., *Flavobacterium longum*, and Rhizobiaceae were enriched and *Stenotrophomonas maltophilia* and *sul*1 significantly increased [80].

Among PE, PET, PBAT, PLA and PBAT/PLA MP mixture and gravel incubated in a WWTP effluent in a not specified geographic site in China for 30 days, PE and PET became more hydrophobic and porous because of degradation. The carbonyl index (CI), defined as the absorbance ratio of the carbonyl moieties and methylene moieties, increased for the BDPs. PCoA of shotgun metagenomic data showed that the microbial populations in MPs and gravel were distinct from each other and from those in water. Pathogens *P. aeruginosa*, *S. enterica*, *S. aureus*, *E. coli*, and *Mycobacterium tuberculosis* were more abundant in the plastisphere and ARGs were 4 to 5 times higher in the biofilms. In PE *aad*A, *ere*A, located on MGEs and thus prone to HGT, and the rifamycin resistance gene *rbp*A were particularly abundant. The MGE encoded genes *qac*E1, *sul*2, *tet*X2, the aminoglycoside phosphotransferase genes *aph*(6)-Id and *aph*(3")-Ib and the florfenicol resistance gene *flo*R, were detected in PET, PBAT/PLA, and PLA. The number of contigs encoding both ARGs and MGEs was higher in biofilms indicating a more efficient HGT. The health risks of ARGs (HRA) was highest in PET, followed by PE, for the high abundance of the gene *erm*B, a multidrug resistance gene and quinolone resistance. *Candidatus Microthrix* carrying *tet*A48, *kdp*E, and the multidrug resistance gene *rpo*B2 was the dominant host of ARGs followed by *unclassified Burkholderiales* and *unclassified Pseudomonadota*. *Acinetobacter* spp. carrying 44 ARGs, including *aad*A, *ksg*A, and *aph*(3')-I was

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associated to PET and PLA. QS functions were about 2-fold enriched and positively correlated with the HRA in the plastisphere [69].

In the biofilm on plastic debris from the South African Msunduzi River that presented total dissolved solids (TDS) and specific conductivity (SC) well above the permissible limits, shotgun metagenomic highlighted that Pseudomonadota was the most abundant phylum, significantly more than in water, at all the sampling sites. *Pseudomonas* and *Flavobacterium* prevailed in the plastisphere in an industrial area, while *Acinetobacter*, *Acidovorax*, *Limnohabitans* and *Polynucleobacter* in an agricultural area, *Undibacterium* and *Sphaerotilus* in urban areas, and *Klebsiella* spp. and *Zoogloea* in WWTP outlet sites. The alpha-diversity in the plastisphere was lower than in water, possibly for the selective pressure exerted by MPs and associated pollutants but 26 ARG types with 372 subtypes showed an average relative abundance significantly higher than in water. These included *aac(6')-Im*, the aminoglycoside nucleotidyltransferase gene *aad*A, *bac*A, the beta-lactamases *car*O, *cfx*A2 and *cfx*A6, the ciprofloxacin phosphotransferase *crp*P, *dfr*A1, *dfr*A14, *dfr*B2, *dfr*A2d, *qac*H, *fos*A, *fos*A5, *fos*C2, *fos*X, *ros*A, *van*R, *van*S, *ere*A, *erm*F, *lnu*C, *mac*A, *mac*B, *msr*E, the MLS resistance genes *vat*, *vat*F and *vga*C, *rpo*B2, *abe*M, *abe*S, *acr*B, *ade*B, *ade*F, *ade*K, *ade*N, *ade*R, *mdf*A, *mex*A, *mex*B, *mex*D, *mex*F, *mex*J, *mex*L, *mex*N and *mex*T, *ugd* (*pmr*E), *arr*, *sul*1, *sul*2, *tet*34, *tet*39, *tet*40, *tet*A, *tet*O, *tet*R, *tet*W, *tet*X, and the triclosan resistance genes *tri*A, *tri*B, and *triC*. The ARGs were mostly associated with plasmids and phages and positively correlated with Pseudomonadota, Bacteroidetes, Actinomycetota, Bacillota, Cyanobacteria, Verrucomicrobia, Chloroflexota, Planctomycetes and Nitrospirae. Among water quality parameters, pH was associated to mupirocin and nitroimidazole ARGs, salinity to mupirocin, nitroimidazole and rifamycin ARGs, TDS and SC to mupirocin, diaminopyrimidine, fosfomycin, diaminopyrimidine, β-lactams and tetracycline ARGs. SC was also positively correlated with nitroimidazole ARGs [81].

Long-read metagenomics with the Nanopore sequencing technology (Oxford Nanopore Technology, UK) showed that in the biofilms developed during 30 days on MPs and rocks deployed in situ and in water samples in the Jiuxiang River and in the Taihu Lake (China), the alpha-diversity in the plastisphere was the highest, followed by that on the stone biofilm. The genera *Variovorax*, *Rubrivivax* and *Thauera*, found to contribute to tetracycline ARG selection, were enriched in the biofilms and ARG were more abundant in the MP biofilm than in water and rock with the enrichment of *mexF* and a class B beta-lactamase and *dfrA* and *ompR* uniquely detected. The plasmid encoded ARGs were more abundant on MPs as well as genes related to plasmid activity, DNA integration, transposition and adhesion thus indicating a higher HGT efficiency. The most prevalent ARGs were rRNA adenine methyltransferases, resistance to elfamycin and to tetracyclines associated to the taxa *Herbaspyrillum* and *Limnohabitans* [82].

At a WWTP outlet site in the Yangtze river (China) the amounts of MPs were maximum, followed by those in the estuary and those at upstream sites, smaller particles of 0.1-0.5 μm predominated and were found also downstream while bigger particles were only present there and not in other sites, PE, PP, PS and PA were identified with PE found also in other sites and fibrous MPs were enriched constituting about 70% of the MPs downstream. The intracellular ARGs determined by HT-qPCR were twice more abundant than upstream and were positively correlated with intracellular MGEs transposases, plasmids, *tnp*A, IS91, *tnp*A7 and ISI247 and the extracellular ARGs were about 2.000-fold higher than upstream. Intracellular ARGs *aph*-VIII and bla_{svh11} were detected only in the plastisphere and extracellular ARGs *bac*A, bla_{GES}, *cml*A1, *dfr*A1 and *qnr*S2 were introduced. Metagenome analysis allowed to identify 12 contigs with high risk ARGs adjacent to MGEs, 8 of these were associated to MPs and 5 to the WWTP discharge site with the association *sul*1 and *tnp*A as the most frequent. The intracellular ARGs were positively correlated mostly with the fiber shape of MPs followed by fragments. *Pseudomonas* genus most frequently carried *bac*A, the three genera, i.e. *CAMDGX01*, *PHCI01* and *Shewanella* associated with fibrous MPs carried *bac*A, *nov*A, *mex*F, *mcr*-4,3 and $bla_{OXA-541}$ and the genera *Pseudomonas* and *Serratia* associated with fragments carried *bac*A, *arn*A, *mex*B, the *P. aeruginosa* efflux pump component *mux*B, *aac*(6')-Ic and the aminoglycoside

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efflux pump *acr*D. Quantitative microbial risk assessment (QMRA) indicated that the infection risk by priority risk AMR pathogens was significantly increased by the WWTP effluent [83].

Some of the reviewed studies established associations of specific ARGs with bacterial hosts in plastic debris from freshwater and these associations shed light on the identity of the main ARG donors and partners in HGT with the possibility to trace their origin based on knowledge on their typical habitats. The established ARG-bacteria associations are reported in Table 1.

Table 1. ARGs and most probable or confirmed bacterial hosts identified in plastic polymers in freshwater with polymer types and study sites.

Plastic polymer	Site	Bacterial host	ARG	Reference
PBAT, PET	microcosm	*Afipia,* Rhizobiaceae *Bacillus* spp. *Gemmobacter, Conexibacter, Lamia*	*qnr*S *sul*2, *bla*Q *tet*A, *tet*C, *tet*X, *ere*B	[39]
PE, PP, PBD	Ganjiang river (China)	*Streptococcus mitis*	*erm*F, *erm*B	[44]
Mixed polymers	Huangpu River (China)	*Afipia* spp.	*aac*(2′)-*I*, *arr*, *cat*, *mex*I, bla_{TEM-1}, *tet*V	[45]
PVC, PLA	freshwater microcosm with tetracycline	*Pseudomonas* spp., Flavobacteriaceae, Actinobacteria	*tet*A, *tet*C, *tet*M, and *tet*X	[21]
PET, PVC	freshwater microcosm with tetracycline	Genera *Pseudomonas, Solobacterium, Achromobacter, Aeromonas, Beggiatoa, Propionivibrio,* and *Paludibacter*	*tet*A, *tet*C, *sul*1, *tet*O	[48]
Mixed polymers	Haihe River (China)	*Enterobacter cloacae*	*tet*G	[49]
APSs	Bracciano Lake (Italy)	*Morganella morganii*	*tet*C, *sul*1, *sul*3, *cml*A1, *cmx*A, $bla_{CTX-M-01}$, $bla_{CTX-M-02}$	[51]
Mixed polymers	Mondego river (Portugal)	*E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella pneumonia, Shigella* spp.	*aac*A4-*cr*, *qnr*S, *qnr*B, *qnr*VC, bla_{CTX-M}, $bla_{CTX-M-15}$, $bla_{CTX-M-32}$, $bla_{CTX-M-55}$	[77]
PE, PP	Poyang Lake (China)	*A. veronii* *Arthrobacter* spp.	bla_{OXA-12}, *cph*A6, *cph*A8, *cph*A3 *mds*B, *nov*A, *rph*A, *emr*K	[23]
BDPs	Lakes in Wuhan (China)	*Riemerella anatipestifer* *Vibrio campbellii* *V. cholerae*	*nov*A, *mac*B, *mds*B, *sav1866*, *tae*A, *arn*C, *mfd* *nov*A, bla_{CRP}, *tae*A, *pmr*E, *tet*4 *pbp1a*, *mur*A, *efr*A, *mtr*A, *tet*PB	[57]

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Mixed polymers	Lake water	*Lysinibacillus* spp., *Exiguobacterium acetylicum*, *K. pneumoniae*, *K. oxytoca*, *K. michiganensis*	*bla*TEM (shared by all bacteria), *bla*SHV, *ade*A, *tet*A, *acr*B, *sul*1, *mec*A, *tet*W, *acr*F, *cmx*A, *sul*2	[58]
Mixed polymers	Red River (Vietnam)	*Aeromonas* spp.	*bla*TEM, *bla*SHV, *bla*CTXM	[61]
PHA	Central Lake (China)	Proteobacteria	*bacA*, *mecA*, *qacA*, *tolC*, *dfrA1*, *mphA*, *mphB*	[64]
Mixed polymers	treated wastewater microcosm	*Stenotrophomonas maltophilia*	*sul*1	[80]
PET, PLA	Treated wastewater	*Candidatus Microthrix* *Acinetobacter* spp.	*tet*A48, *kdp*E, *rpo*B2 44 ARGs including *aad*A, *ksg*A, *aph*(3′)-I	[69]
Mixed polymers	Jiuxiang River and Taihu Lake (China)	*Variovorax*, *Rubrivivax*, *Thauera* *Herbaspyrillum*, *Limnohabitans*	ARGs for tetracycline ARGs for MLS, elfamycin and tetracyclines	[71]
Not specified	Urban lake in Chengdu (China)	Gammaproteobacteria, Pseudomonadota, Acidobacteriota, Actinomycetota	*bac*A, *sul*1	[66]
	Urban river trait Chengdu (China)	Deltaproteobacteria, Desulfobacterales	*sul1*, *sul2*, *acrB*, *ant*(2″)-Ia	
	Rural lake Chengdu (China)	Gammaproteobacteria	*sul 1*, *sul 2*, *smeE*	
Not specified	Yangtze river (China)	genera *CAMDGX01*, *PHCI01*, *Shewanella* genera *Pseudomonas*, *Serratia*	*bac*A, *nov*A, *mex*F, *mcr*-4,3, *bla*OXA-541 *bac*A, *arn*A, *mex*B, *mux*B, *aac*(6′)-Ic, *acr*D	[83]

APSs, Artificial Plastic Substrates; PBAT, poly butyleneadipate-co-terephthalate; PBD, polybutadiene; PE, polyethylene; PET, polyethylene therephtalate; PP, polypropylene; BDPs, biodegradable plastics; PHA, polyhydroxyalkanoate; PLA, polylactic acid; PVC, polyvinylchloride.

4.2. ARG Presence in the Plastisphere In Seawater

An early study on the occurrence of ARGs in the plastisphere in seawater regarded the analysis of metagenome datasets, 12 from MPs, 12 from macroplastics and 16 from seawater, collected in the North Pacific Gyre and available from the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/, accessed on 2 July 2025). ARGs were found in all the plastic-associated microbial communities but only in one fourth of the water samples. Micro- and macroplastics harbored a more diverse microbiota than water and a relative abundance of ARGs about one order of magnitude greater with c.a. 10^{-3} copies per 16S rRNA gene copy. Sixty-four ARGs for resistance to thirteen antibiotics were identified on plastics, among which MDRGs and genes for aminoglycoside resistance were the most abundant not distinct in diversity and copy number for different plastic particle sizes. ARGs were significantly associated with Flavobacteriaceae, Cyanobacteria subsection III family l, Sneathiellaceae, and Flammeovirgaceae. Those identified were *aac(3)*-I, *aad*E, *aac(2′)*-I, *aph(3′)*-I, *bac*A, the bacitracin resistance gene *bcr*A, *bla*TEM

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variants 1 and 185, *bla*$_{VEB}$ variants 1, 2, 3, 4, 5, 6, and 8, *cat*, *fos*X, *fos*A, *ros*A, *ksg*A, the macrolide efflux genes *mac*A and B, *vat*A, C and D, *mex*B, E, F, I, T, W, *omp*R, *acr*B, the MDRGs *mdt*K, *bpe*F, *acr*A, *tol*C, *opr*C, *ceo*B, *sme*B, *ade*J, *ade*B, *cme*B, *mdt*B, *mdt*C, *mdt*F, *amr*B, and *cpx*R, and ARGs *tet*A, *tet*C, *tet*P, *tet*V, *tet*34, *tet*35, *tet*39, *tet*41, *van*B and *van*R. Genes for beta-lactam and tetracycline resistance showed co-occurrence in the correlation network [84].

Among 37 bacteria isolated, from macroplastic samples collected at the intertidal zone of Vestland county, a, isolates of the fish pathoges *A. salmonicida* were resistant to ampicillin and isolates of the opportunistic human pathogens *M. morganii* and *A. beijerinckii* were resistant to at least three classes of antibiotics. Whole genome sequencing (WGS) highlighted the presence of class C beta-lactamases and chloramphenicol acetyltransferase *cat*B in all the isolates, and class B2 metallo-beta-lactamase *cph*A in three *A. salmonicida* isolates, with a new variant in two cases. Moreover, all *Aeromonas* isolates carried the quinolone resistance gene *qnr*A. The *A. beijerinckii* isolate harbored new variants of class A beta-lactamases, an aminoglycoside acetyltransferase and *cat* [85].

PE fragments of 5-10 cm retrieved from a stream in Vallone Casteldaccia (Italy) and from seawater in front of the stream was analyzed by metataxonomic analysis. Hundreds of operational taxonomic units (OTUs) were detected in all samples and in PE from freshwater and the surrounding water their number was similar while in PE from seawater the number of OTUs was about four-fold higher than in water. Bacterial phyla were not enriched in PE MPs compared to water except for Dadabacteria, Elusimicrobia, and Hydrogenedentes in MPs from seawater. The PE from the sea and seawater formed a separate cluster from PE from freshwater and the surrounding water in PCoA analysis. The ARGs *erm*B, *tet*A, *tet*W, *sul*2, *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *qnr*S and *int*1 were sought by PCR and the gene *bla*$_{TEM}$ was detected in all samples, *ermB*, *qnrS*, *sul*2, and *tetA* on PE from both environments, *bla*$_{CTX-M}$ in PE from seawater and *tet*W in PE from freshwater [86].

It was demonstrated that the leachate from PVC, which contains organic, inorganic substances and zinc, can enrich ARGs in coastal surface waters microcosms in absence of PVC solid particles. This leachate was prepared by acid washing plastic containers and tested at two concentrations during an incubation of six-days. An increase of ARG abundance, but not diversity, was observed with increasing leachate amount. Aminoglycoside ARGs, mainly efflux pumps, target modification and the beta-lactamase *amp*C, and MDRGs were enriched while MLS ARGs decreased. ARGs were associated to the genera *Tritonibacter*, *Alteromonas*, a genus that can grow in plastic leachate, and *Alcanivorax*. Among these, *Alteromonas* can transmit integrative conjugative elements (ICEs) to human pathogens [87].

In a study carried out at two sites in the coast of Barcelona (Spain) where waters from WWTPs, two rivers and recreational plants merge, in MPs collected during two cruises in 2022 the 16S rRNA gene copies were of the order 10^6/mm^2. The genes *sul*1, *tet*W, *bla*$_{TEM}$ were detected in 3 to 5 of six MP samples collected in each site at levels ranging from tens to hundreds of copies/mm^2. The number of positive samples and ARG levels were lower for MPs from the sediment [88].

In PE, PP, PS and PVC MPs deployed in a marine environment not heavily impacted by human activities close to Busan City (South Korea) for 102 days a strong biofilm formed after 63 days particularly on PE and PP. Metataxonomic analysis showed a higher microbial diversity in the MPs than in water with the enrichment of Acidimicrobiia and Planctomycetes while Anaerolineae were present only in MPs. The bacterial genera detected on MPs were *Methylotenera*, involved in EPS formation, *Granulosicoccus*, *Maritimimonas*, *Ketobacter*, *Pseudahrensia*, *Aquibacter*, and *Aquimarina*. MP ARGs were significantly more abundant in water except for tetracycline ARGs including *tetM* and *tetS*. ARGs detected in MPs were in order of abundance *tetA*, *sul1*, *tetC*, *ermB*, *tetQ*, and *qnrS*. The genes *tet*A and *sul*I were more abundant on PVC. The MGE *intI1* and ARGs *ermB*, *sul1*, and *tetC* increased on PE, PP and PS until day 63 and decreased later while *tet*A increased until day 102. Network correlation analysis suggested that *Coxiella* spp. and *Pseudahrensia* spp. were the possible hosts of *tet*A and *tet*Q, respectively, while the genera *Fuerstia*, *Methylotenera*, *Halioglobus*, *Ahrensia*, *Rubritalea*, and *Algibacter* were potential hosts of *erm*B [89].

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Shotgun metagenomic showed that in two coastal sites of the Tyrrhenian Sea (Italy), one little impacted by human activities and the other with high anthropogenic pollution, and in a pelagic site the genera *Vibrio* and *Alivibrio* spp., *Shewanella* and *Buchnera* spp. were the most abundant components of the biofilm both on natural organic particles and in MPs sampled from surface waters. The ARG *qnr*S was only present in particles and among MAGs associated to MPs one identified as Rhizobiales contained *bac*A, a *Photobacterium* spp. encoded two copies of *tol*C, *acr*B and *tet*34, and *Pleurocapsa* spp. encoded *vat*F next to MGEs [90].

MPs sampled at two sites close to the sea shore in Reunion Island (Madagascar), one receiving human pollution and the other almost exempt from it in metataxonomic analysis showed a lower microbial diversity and a different abundance of genera than water with cultivable bacteria counts of about 10^7 CFU/g. Sixteen among 105 isolates were assigned by Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) to the genera *Bacillus*, *Enterococcus*, *Pseudomonas* and *Pantoea* and showed non-intrinsic resistance to penicillin, ampicillin and ticarcillin [91].

Coral reefs formed by scleractinian corals are a complex ecosystem hosting at least 25% marine organisms found to be polluted by MPs, mainly PET and cellophane (CF). PET MPs were submerged for 18 days in two coral reef sites in the Hainan province (China) where the antibiotics trimethoprim, sulfaquinoxaline, florfenicol, norfloxacin, and enrofloxacin were detected, with also ofloxacin and ciprofloxacin in one site. Biofilm formation in the MPs was confirmed by SEM and a metataxonomic analysis highlighted a microbial population distinct from that of water and including the genera *Acinetobacter*, *Desulfovibrio*, *Desulfopila*, IheB3-7, *Lutibacter*, *Hellea*, *Halarcobacter*, *Candidatus*_Falkowbacteria, *Neptuniibacter*, and *Rhodovulum*. Seventeen genera and five ARGs showed positive correlations in the plastisphere among which *sul*2, that was the most abundant, correlated with nine bacterial genera, and the less abundant ARG was *qnr*B. All bacterial genera were correlated with at least two ARGs and *Vibrio* spp. with *sul*1 [92].

In the touristic and fishing coastal areas of Monastir and Mahdia (Tunisia) 66 isolates were obtained from MPs separated by density from the sediment. These were distinguished by 16S - 23S rRNA internal transcribed spacer fingerprinting and assigned by 16S rRNA sequencing to the genera *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Shewanella*, *Aeromonas*, *Vibrio*, *Stutzerimonas*, *Exiguobacterium* and *Enterobacter*. Phenotypic antibiotic resistance testing provided 41 MDR profiles comprising resistance to beta-lactams, glycopeptides, aminoglycosides, rifampicins, polymyxins and quinolones. A strain of *S. arctica*, a species able to form biofilms, showed the highest level of MDR and resistance to beta-lactams for the presence of the *bla*$_{\text{TEM}}$ gene that occurred in 10% of the isolates [93].

PE and PVC panels were deployed for up to 12 months at 5 m and 20 m depth in two bays of the Ross Sea (Antarctica), one exposed to human pollution and the other not impacted by human activities, during an Italian Antarctic campaign (2017–2018). The plastisphere biofilm was quantified by a staining technique and bacteria were isolated and identified by sequencing a region of the 16S rRNA gene. The PVC biofilm was more abundant for the human impacted site and at the not impacted site was less abundant than that on PE. AMR was determined phenotypically towards three antibiotic classes The highest ARB number in PVC was unexpectedly found at the not impacted site at 20 m depth. On the PE panels deployed in the site impacted by human pollution ARB represented 70% and 90% of the isolates from panels submerged at 5 and 20 m depth, respectively, while at a control site without human impact ARB percentages were about halved. The multiple antibiotic resistance (MAR) index, i.e. the number of resistances on the number of antibiotics tested, was generally higher for PE than PVC but for the latter a higher MAR index was calculated in the not impacted bay. For PVC resistance to quinolones, lincosamides, and rifamycins mostly influenced the MAR index while for PE reistance to beta-lactams, cephalosporins, oxazolidinones, and glycopeptides prevailed. Also, seawater contained ARB that accounted for 62/72% isolates thus showing the presence of a resistome in an environment not contaminated by antibiotics [94].

Mangroves are a source of pollutants directly connecting land and sea for their capacity to retain MPs, and associated antibiotics, pathogens and ARGs [34]. These ecosystems contribute MPs to

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aquatic organisms, as shown by their isolation from a mudskipper fish (*Periophthalmus waltoni*) in Southern Iran [95]. PP, high-density PE, PS, PET, and PCL MPs were buried in mangrove soil in three sites of the Guangdong Province (China) for 30 days and reached about 10^8 ARG copies/g, more abundant than in sediment in two sites. The most frequently detected ARGs were *sul*1 and *sul*2 and, in a site with higher human impact, *erm*F. The gene *msb*A was also enriched. PET showed the highest ARG enrichment, PP and PE constantly showed high ARG abundance and PS showed a *tetA* and *tetT* enrichment higher than other MPs. The gene *intI*2 was the most abundant MGE gene in PLC in one site. ARGs on MPs were significantly correlated with *intI*1 and *intI*2 and with human impact and environmental factors. Proteobacteria and Firmicutes predominated in most MPs and the genera *Acinetobacter*, *Bacillus*, *Desulfobulbus*, *Fonticella* and *Vibrio* were positively correlated with ARGs [96].

In 42 coastal mangrove sites including urban areas, aquaculture areas and natural reserves along the Southern coastline of China between April and November 2021, PP, PE, polyadiohexylenediamine (PA66), polymethyl methacrylate (PMMA), PS, PET, and PVC debris were identified. The ARGs *sul*2 was the most abundant in the plastisphere especially in the aquaculture zones, and *tetA*, *tetT*, *sul2*, *msbA*, *ermF* were detected by qPCR with an absolute abundance of the order 10^6 – 10^9 copies/g with a total ARG level of the order 10^{17} copies at all sites. ARG abundance was higher in the urban area where *ermF* predominated. Among MGEs, *intI*1 was detected at absolute abundance of the order 10^4 -10^6 copies/g. Most ARGs except *msbA* were positively correlated with *intI*1 indicating the potential for HGT spread [97].

After incubation for 1 month of PE, PS, and PVC MPs in the National Mangrove Reserve in Zhangzhou (China) a significant separation was observed between the bacterial communities in MPs and soil and among the three MPs with higher abundance of the Proteobacteria families Sphingomonadaceae and Rhodobacteria and of ARGs, mainly MDRGs, *bacA* and *bcrA* in MPs. High-risk ARGs of Ranks I and II, defined according to the classification system of Zhang et al. [63] were more abundant in the plastisphere with a predominance of quinolone resistance, followed by MDR and aminoglycoside resistance. The relative abundance of plasmids, integrons and insertion elements, was higher for MPs and these were positively associated with ARGs. MPs, and particularly PS, showed a higher relative abundance of the pathogens *S. aureus*, *P. aeruginosa*, *Listeria monocytogenes*, *M. tuberculosis*, *Bordetella pertussis*, *E. coli*, and *Salmonella enterica*. The first four species predominated and were positively correlated with high risk ARGs. Contigs of ARG-carrying plasmids were more abundant on MPs and particularly on PE showing HGT potential. ARGs, adhesion virulence factors and MGEs were colocalized in pathogenic bacteria, mostly for *Pseudomonas* spp. and only in MPs [98].

Some of the reviewed studies established associations between ARGs and bacterial hosts in plastic debris in seawater that are reported in Table 2.

Table 2. ARGs and respective most probable or confirmed bacterial hosts identified in plastic polymers from seawater with polymer types and study sites.

Plastic polymer	Site	Bacterial host	ARG	Reference
Mixed polymers	Vestland county, Norway	*A. salmonicida*, *M. morganii*, *A. beijerinckii* *A. salmonicida* *Aeromonas* spp. *A. beijerinckii*	class C beta-lactamases, *cat*B *cph*A *qnr*A class A beta-lactamase, aminoglycoside acetyltransferase, *cat*	[85]
PBAT, PET	microcosm	*Labrenzia*, Vicinamibacteraceae	*tet*A, *bla*Q	[39]

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PBAT, PET	microcosm	*Labrenzia*, Vicinamibacteraceae, Acidobacteriota, *Coxiella*, *Croceibacter*, *Tumebacillus*	*qnr*B	
Not specified among PE, PP, PS and PVC	Busan City (South Korea)	*Coxiella* spp. *Pseudahrensia* spp. Genera *Fuerstia*, *Methylotenera*, *Halioglobus*, *Ahrensia*, *Rubritalea*, *Algibacter*	*tet*A *tet*Q *erm*B	[89]
Mixed polymers	Tyrrhenian Sea (Italy)	Rhizobiales *Photobacterium* spp. *Pleurocapsa* spp.	*bac*A *tol*C, *acr*B, *tet*34 *vat*F	[90]
PET	Coral reef, Hainan (China)	Nine bacterial genera *Vibrio* spp.	*sul*2 *sul*1	[92]
Mixed polymers	Monastir, Mahdia (Tunisia)	*Shewanella arctica*	bla_{TEM}	[93]

PBAT, poly butyleneadipate-co-terephthalate; PE, polyethylene; PET, polyethylene therephtalate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride.

4.3. ARG Presence in the Plastisphere in Estuaries and Brackish Waters

Estuaries are characterized by salinity gradients and the presence of multiple pollution sources. PS, PP, PE, PET and PVC macroplastics sampled at seven sites of the Yangtze Estuary (China) were found to harbor the genes *intI*, *sul*1, *tet*A, *tet*W, *aac(6')*-Ib, *chl*, determined by qPCR, at higher levels than sediment and water. The ARGs ranged between multiples of 10^6 copies/g and 10^9 copies/g among the sites. The average abundance of *intI*1 was of about 10^8 copies/g in the MP biofilm. The copy number of the 16S rRNA gene ranged between multiples of 10^9 to 10^{11}/g in plastic biofilms, explaining the high absolute abundance of ARGs in the plastisphere. The same polymer showed different biofilm communities in different sites and the correlation between antibiotic concentrations and the absolute abundance of the corresponding ARGs was not significant. All ARGs were negatively correlated with salinity [99].

Among plastic polymers deployed for 153 days at the interface water/sediment in the Laguna Madre, a coastal lagoon and estuarine area in Mexico PHA presented a higher relative abundance of ARGs than water, ceramic used as control and PET with *dfr*E among the most abundant genes and the multidrug efflux pump *crp*, *macB* and *pmr*E among the enriched genes. The *sul* genes were present only in water and PET. MAGs and the reconstructed resistomes confirmed that the identified ARGs were abundant in PHA. In addition, the ARGs *bac*A, *fos*X and *vat*B were found in some samples. Among the isolates from PHA submitted to WGS two close neighbors of the *Bacillus cereus* and *B. thuringiensis* groups harbored ARGs for resistance to 10 and 20 antibiotic classes, respectively, and were phenotypically resistant to ampicillin, carbenicillin, cephalothin, and penicillin, vancomycin and bacitracin with intermediate resistance to clindamycin and erythromycin [100].

MPs collected near the estuaries of the Yangtze, Sheyang, Guanhe and Xinyi rivers (China) harbored a number of 16S rRNA gene copies of the order 10^9 and *sul*1 was the intracellular ARG mostly enriched among those searched by qPCR, namely *tet*A, *tet*M, *tet*X, *sul*1, *sul*2, bla_{TEM}, bla_{NDM-1}, *ere*A, and *erm*B, that were all detected. Extracellular ARGs obtained from the filtrate of the cell suspension used to extract intracellular DNA showed a higher enrichment on MPs, possibly influenced by the selective pressure and aging. The number of intracellular and extracellular ARG were in the order of 10^8 copies/g and 10^7 copies/g, respectively, and decreased in the sites more distant from the coast possibly for the increased salinity. The genera *Bacillus*, *Lactobacillus*, *Pseudomonas*,

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Streptococcus and 32 bacterial species were positively correlated with ARGs. The genes *bla*$_{NDM-1}$, *tet*A, *tet*X, *sul*1 and *sul*2 correlated with Proteobacteria and *bla*$_{TEM}$, *erm*B, *ere*A, *tet*X, and *sul*1 with Bacteroidota. Redundancy analysis highlighted a positive correlation of intracellular *tet*A and *sul*1 with pH and a negative correlation of *sul*1 and *sul*2 with total nitrogen. The extracellular ARG t*etM*, *sul1*, *bla*$_{TEM}$, *bla*$_{NDM-1}$ were negatively correlated with pH and positively correlated with total nitrogen and total phosphorous thus showing a connection with nutrient availability [101].

In Haihe Estuary connecting the Haihe River to the Bohai Sea (China), PET MPs of about 3 mm size were incubated sequentially at three sites progressively closer to the sea thus determining a "mobile plastisphere". This was always dominated by Proteobacteria that represented more than 50% of the microbiota identifed by metagenomic analysis and Bacteroidota followed at about half percentages. The phyla Planctomycetota, Verrucomimicrobia, Gemmatimonadetes, and Nitrospirae were enriched. The Thermodesulfobacteria increased during the transfer towards increased salinity while other phyla decreased but diversity at the species level increased. MDRGs and ARGs for resistance to glycopeptides, beta-lactams, pleuromutilin, fluoroquinolones, triclosan, aminoglycosides, rifamycin, and bicyclomycin decreased in the sea and in the site just upstream the number of ARG subtypes was higher. Beta-lactam and fluoroquinolone ARGs increased at the second site and the number of subtypes remained stable at site 3. In all cases the number of subtypes followed the order beta-lactams > multidrug > glycopeptides > MLS. Among 814 ARG subtypes detected 655 persisted at all sites. Proteobacteria were the main ARG hosts and at downstream sites Bacteroidota hosts increased. Moreover, at species level bacterial ARG hosts were different in the mobile plastisphere and in the sea. Pathogenic ARG carriers detected at all sites were *E. cloacae*, *K. pneumoniae*, *M. tuberculosis* and *V. parahaemolyticus* that decreased in the travel and were not detected in the sea. Other detected pathogenic ARG hosts that also decreased were *P. aeruginosa*, *Burkholderiales* and *Y. enterocolitica*. The PPR model indicated that estuarine environments reduce the ARG risk deriving from land sources. Nevertheless, the AMR risk at site 3 was still high and could be transferred to the marine ecosystem [102].

At four sites in a pharmaceutical and chemical industrial park in the Yangtze River Delta region, China, rubber, polyisoprene chlorinated (PLC) and PE were detected in groundwater samples obtained from wells, representing about 61%, 9%, and 8% of the MP abundance, respectively. Rubber and PLC were positively correlated with the antibiotics roxithromycin, sulfamethazine and clindamycin. PE and PLC were positively correlated with *sul*1, *erm*F and *intI*1 while rubber and PA were negatively correlated with the ARGs. The microbial groups identified by metataxonomic analysis, namely Comamonadaceae, *Acinetobacter*, *Pseudomonas*, *Simplicispira*, and *Proteiniphilum*, showed significant positive correlations with ARGs [103].

In the fresh and brackish waters adjacent to the metropolitan areas of Tokyo, Saitama and Chiba (Japan) the highest level of MPs was near the outlet of the Arakawa River and the most abundant MPs were PE and PP followed by PA and smaller amounts of PS, PET, PVC and PU. Shotgun metagenome analysis highlighted a lower diversity of bacteria on MPs than in water with the selection of bacterial populations including the genera *Exiguobacterium*, *Eubacterium*, *Dolichospermum*, *Anabaena*, *Gloeothece, Nodularia* and *Planktothrix*. Among 1110 ARGs detected, 38 were exclusively found on MPs and with different relative abundance in the Tone River and the Arakawa River. The most abundant ARGs in MPs were the MDRG *rsm*A followed by *qac*G, *van*T, *van*Y, *van*W, *van*H, *bla*$_{OXA-53}$, *bla*$_{OXA-912}$, and *bla*$_{IND-6}$ and ARG subtypes present only in MPs were *bla*$_{OXA-912}$, *bla*$_{OXA-726}$, *fos*A8, the aminoglycoside resistance genes *rmt*F and *apm*A. MGEs, namely subtypes of *is_tn*, phage genes, such as *rev*, *rev*_3, phage_integrase, *tnp*R, *Relaxase*, *Int_CTnDOT*, *Tn916*, *Phage_GPA* were more abundant in the plastisphere than in water. Correlation network analysis showed that the genera *Citrobacter*, *Aeromonas*, *Sulfitobacter*, and *Lacinutrix* were potential hosts for *bac*A, *Acr*AB-*Tol*C, a mutated *mar*R conferring resistance to ciprofloxacin and tetracycline, the *acr*AB inducer *mar*A, *van*G, *ade*F, *qac*G, *van*H, and *K. pneumoniae* was the probable host of a mutated *kpn*F efflux pump conferring resistance to most antibiotics [104].

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PE, PP, and PS MPs with sizes 3 mm, 200 μm, and 30 μm deployed for two months in the Xinglin Bay (China) had a zeta potential between -48 mV for particles of 30 μm and -5 mV for particles of 3 mm and the specific surface area calculated as Brunauer-Emmett-Teller was up to ten-fold larger for the 30 μm MPs. Indeed, the biofilm detached from the smaller particles showed an OD_{595} five to nine-fold higher than that from the 3 mm MPs. The most abundant ARGs in the MP biofilms encoded MDR, bacitracin and sulfonamide resistance and the ARG profiles changed with MP particle size for PE and PP. Shotgun metagenomic showed that the composition of the microbiota did not vary significantly with the MP particle sizes and the dominant bacterial groups Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes presented a higher diversity than in water. The pathogens *P. aeruginosa*, *L. pneumophila*, *K. pneumoniae*, *M. tuberculosis*, *A. hydrophila*, *A. baumannii*, *B. pseudomallei*, and *Brucella melitensis* were more abundant in the MPs. The co-occurrence of *intI*1 with genes *aac*(6')-I and *bac*A and *ICEKpn342*-1 and *intI*1 with *mexT* on the same contigs was observed. The zeta potential and contact angle were negatively correlated with ARG abundance. The risk score calculated by taking into account the occurrence of ARGs, MGEs and pathogens increased with particle size for PS and PP while the opposite was observed for PE [105].

PE, PLA, PS, PVC, MPs and Tetra Pak incubated in the same bay for 50 days showed a higher risk of ARG transmission compared to wood, rock and glass as demonstrated by combining single-cell spectroscopy and HT-qPCR carried out in a SmartChip Real-time PCR system with 384 primer pairs for ARGs, taxonomic markers, MGEs, and the 16S rRNA gene. The biofilm on wood was the most abundant but positive co-occurrence correlations were more numerous and correlation networks less complex among microbial groups in MPs. Metataxonomic analysis of bacteria and fungi and the neutral community model (NCM) indicated that deterministic processes like species interaction and niche differentiation influenced the microbial assembly on MPs that presented a ten-fold higher health risk for the proportion of metabolically active hosts of high-risk ARGs. D_2O-labeled single-cell Raman spectroscopy applied to the microbial communities challenged with ciprofloxacin and the last-resort antibiotics colistin and meropenem showed that ARB for these antibiotics were more abundant on MPs than on natural surfaces. Tetra Pak, PS, and PLA showed a higher abundance cyprofloxacin-resistant bacteria, PLA a higher abundance of meropenem-resistant bacteria and all MPs a higher abundance of colistin-resistant bacteria. Moreover, the colistin resistance gene *mcr*1 was connected to 32 bacterial genera, some of which positively correlated to the MGEs *mob*A, *Inc*Q_*ori*T, and *orf* 37-IS26, indicating HGT potential. The genotypic health risk index (GHRI) for a ARGs that depends on abundance in the environment, mobility, association to pathogens and resistance to antibiotics of clinical relevance, was highest for Tetra Pak followed by wood and then the MPs and it was mainly influenced by ARGs for aminoglycosides and tetracycline and MDRGs. The integrated health risk index (IHRI) that takes into account biofilm biomass, metabolic activity, ARGs present, phenotypic AR, and pathogen abundance was nine to twenty-fold higher for Tetra Pak, PLA, and PS mainly influenced by phenotypic AMR as the dominant factor followed by surface hydrophobicity [106].

Some studies established associations of ARGs and bacterial hosts in plastic debris in estuarine waters as reported in Table 3.

Table 3. ARGs and respective bacterial hosts identified in plastic polymers from estuarine and brackish waters with polymer types and study sites.

Plastic polymer	Site	Bacterial host	ARG	Reference
Mixed polymers	Laguna Madre (Mexico)	*Bacillus cereus*	ARGs for 10 antibiotic classes	[100]
		B. thuringiensis	ARGs for 20 antibiotic classes	

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Mixed polymers	Yangtze, Sheyang, Guanhe and Xinyi rivers (China)	Proteobacteria	*bla*NDM-1, *tet*A, *tet*X, *sul*1, *sul*2	[101]
		Bacteroidota	*bla*TEM, *erm*B, *ere*A, *tet*X, *sul*1	
Mixed polymers	Tokyo, Saitama, Chiba (Japan)	Genera *Citrobacter*, *Aeromonas*, *Sulfitobacter*, *Lacinutrix*	*bac*A, *acr*AB-*Tol*C, mutated *mar*R, *acr*AB inducer *mar*A, *van*G, *ade*F, *qac*G, *van*H	[104]
		K. pneumoniae	*kpn*F	

4.4. The Role of Viruses in ARG Transmission in the Plastisphere

A role for transduction in the HGT of ARGs in plastic debris was indicated by their presence in the genomes of viruses detected in the plastisphere as observed in a study on PE, PP, PS, PE-fiber (PF), and PE-fiber-PE (PFP) deployed in five sites upstream and downstream from the estuary of the Bei-Lun River (Vietnam) for 30 days. Metagenomic analysis showed that the viral community was distinct among polymers but not among sites and the abundance of each virus varied among sites. For example, the circular DNA virus-25 associated to sewage was more abundant in the estuary. Eleven ARGs were detected in viral genomes among which the fluoroquinolone resistance gene *gyr*B4 was the most abundant. Moreover, 90% of the virus associated ARGs were found in PP upstream of the estuary and in PE downstream and in the estuary. The viruses detected in PP harbored *vatB*, *dfrE*, *rpoB*, *rpoC*, *gyrB1* and *gyrB2* while those detected in PE harbored *gyrB3*, *parC*, *parE*, *gyrB4*, and *gyrA*. The PPR model by taking into account the ARGs and virulence factors in the viral genomes indicated that the potential risk related to the MPs was highest for PP and positively correlated with its highly positive z potential that favors the binding of the [29] DNA. Moreover, network correlation showed more numerous interactions between viruses and bacteria on PP [107].

In the biofilm formed during 21 days on PE MPs deployed in the Xixuan Island sea (China) metagenomic analysis showed the presence of 4999 phages of which 609 were associated with the genera *Vibrio* and *Bacillus*. Twenty-eight ARGs encoding resistance to MLS, aminoglycosides, beta-lactams, bicyclomycin, fosfomycin, and multiple drugs were found in the genomes of the DNA viruses while in the RNA viruses the ARG *OmpK37* encoding a beta-lactamase from *K. pneumoniae* was identified [108].

From the analysis of 180 publicly available plastisphere metagenome datasets from different environments 4062 phage contigs were identified, were significantly associated with pathogens and ARB mostly in BDPs and encoded ARGs that can be horizontally transferred [109]. This underlines the importance of further investigating the role of viruses in ARG HGT in the plasisphere in aquatic environments.

5. Effects of MPs on ARG Selection in Aquatic Animals and Fishery Products

5.1. Presence of ARGs in MPs in Aquaculture Farms

MPs colonized by ARB in aquatic environments can directly contaminate fishery products and transmit ARGs or elicit ARG selection in the intestinal tract of the animals but this connection was limitedly investigated. Aquatic animals are one of the main sources of MPs in the human diet since fishes ingest MPs by mistaking them for food and by trophic exchange. Moreover, MPs accumulate in filtering molluscs. In particular, the European Food Safety Authority in a scientific advice on contaminants in the food chain warned that a 225 g portion of Chinese mussels can contain up to 900 MP particles [110]. MP contamination can reach human consumers from different steps of the aquatic trophic transfer in which mussels, clams and crabs are the primary consumers, small fishes the secondary consumers and predatory fishes the tertiary consumers [111]. Fishes more prone to accumulate MPs are those feeding on a wide range of preys compared to those with selective feeding

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[108]. Moreover, MPs showed detrimental synergistic effects with the veterinary antibiotics oxytetracycline, florfenicol, and sulfamethoxazole on *Mytilus coruscus* mussels among which antibiotic accumulation in tissues [112].

Aquaculture is a relevant source of MPs in aquatic environments due to its large use of plastic items such as fishing nets, floats and packaging materials [113]. The effects of MPs in aquaculture are environmental degradation, toxicity and selection of ARGs that ultimately determine a loss of productivity of these systems [114]. Mariculture, i.e. aquaculture practiced near the coasts, is a hotspot of ARG selection for its relevant use of antibiotics often added to the feed. The mariculture effluent, which can contain antibiotic residues in some countries, is discharged directly into the sea where these residues are diluted to sub-lethal concentrations that promote the enrichment of ARGs such as *sul*1, and *sul*2 and the *tet* genes [115].

Antibiotics are adsorbed by the plastic debris as shown by a study on PS, PP, PE, PET and PVC MPs aged for eight weeks in a scallop farm, an abalone farm and in a site without mariculture farms in the Dongshan bay (China). A maximum antibiotic concentration of 26 ng/g was detected in MPs with PP showing the highest adsorption rate for sulfanitran followed by danofloxacin, marbofloxacin, josamycin, tetracycline and doxacycline. The diversity of the MP associated bacteria was higher than in water with a predominance of Proteobacteria and Bacteroidetes and the genus *Vibrio* followed by Firmicutes and Actinobacteria. PCoA indicated the dinstinctness of the MP microbiota from that of water and between the two mariculture farms but not among the MP types. The human health risk quotient (HQ) posed by the antibiotics adsorbed on MPs was derived from the estimated daily intake (EDI) and the acceptable daily intake (ADI) and josamycin in scallops reached an HQ value of risk [116].

In a recirculating mariculture plant in Fujian Province (China), PET fibers were the dominant MP type in biofilter water, recycled water, and fish pond and were released by the biofilter made of this material. The number of copies of the 16S rRNA gene on the MPs from the three compartments was of the order 10^7 - 10^8/g and the absolute abundance of ARGs determined by qPCR was of the order of 10^9 copies/g with *sul*, *tet*, *qnr* and *erm* genes at 3-4 orders of magnitude higher than in water. The genes *tet*X, *qnr*A and *erm*F predominated but *tet*B, *tet*G, *qnr*B and *qnr*S were also detected. The *intI*1 indicator of HGT potential was of three orders of magnitude higher on MPs than in water. The microbial phyla detected, namely Proteobacteria, Bacteroidetes, Planctomycetes, Chloroflexi, Actinobacteria, Firmicutes, Chlorobi, Cyanobacteria, and Spirochaetae were more abundant in MPs and the species richness was higher than in water. Twenty-five bacterial genera were present only in MPs or enriched in MPs and were positively correlated with *intI*1 and at least five ARGs, including *sul*1, *sul*2, *tet*G, *erm*F, and *qnr*S [117].

Similar results were obtained for a recirculating mariculture farm in Yantai (China) in which fibrous PET MPs derived from the filter were the most abundant MPs in all compartments. Cultivable bacteria reached multiples of 10^8 CFU/g in MPs, three orders of magnitude higher than in water, at all sampling points and presented a higher percentage of ARB resistant to chloramphenicol, tetracycline and sulfafurazole with a minority resistant to gentamicin, ciprofloxacin, penicillin and erythromycin. Based on metataxonomic analysis of bacteria grown in presence of antibiotics, the microbial population of ARB on MPs was more diverse than in water with a predominance of Proteobacteria and Bacteroidetes and the genera, *Vibrio*, *Muricauda*, *Ruegeria* and *Sunxiuqinia*. Among these, *Maricauda* carried the highest number of ARGs followed by *Ruegeria* and *Vibrio* and the most frequently detected ARGs were *flo*R, *sul*2 and *bla*$_{SHV}$. Multiresistant bacteria (MARB), among which the pathogen *V. alginolyticus* predominated, were more abundant in MPs as well. The most frequent multiple resistance profile included resistance to tetracycline, sulfurazole, erythromycin and penicillin followed by the same profile plus resistance to chloramphenicol. The integrase gene *intI*1 was detected in 83.8% of MARB isolates and was associated with *aadA*2, *aadA*5, *aad*B, *dfr*A1, *dfr*A27, *ere*A and *arr*2 [118].

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In another study on a mariculture plant, the distribution and relative abundances of ARGs and MDRGs in water and MPs were not found to be significantly different. The most abundant ARG encoded chloramphenicol resistance and the *intI*1 was the most abundant MGE indicator [119].

Oysters, a relevant aquaculture product, accumulate MPs by filtration. Exposure of oysters for 30 days to PE, PP, PET, poly-hydroxybutyrate (PHB) and PLA, and control particles of wood and glass was carried out in a farm in Zhenhai Bay (China). Biomass growth was slower on NBPs but after 14 and 30 days it was comparable to that formed on BDPs and SEM imaging highlighted the formation of EPS. Metataxonomic analysis showed that the microbial diversity on MPs became higher than in water at day 14. The Proteobacteria families Rhodobacteraceae, Comamonadaceae and Pseudomonadaceae, and, at day 30, also Flavobacteriaceae and Sphingomonadaceae, were the most abundant phyla on MPs, and were higher than in water. Biomarker genera were for PE the hydrocarbon-degrading *Alcanivorax*, the polycyclic aromatic hydrocarbon degrader *Erythrobacter* and the potentially pathogenic genus *Tenacibaculum*, for PLA *Hydrogenophaga* and the potential pathogen *Pseudoalteromonas*, and for PP by *Candidatus Amoebophilus*. The relative abundance of *sul*1, *qnr*S and *bla*TEM was lower in MPs than in water due to the higher biomass but the relative abundance of *intI*1 was higher in MPs, indicating a high risk of ARG HGT [120]. Moreover, *P. aeruginosa*, a Priority 1 ARB pathogen [121], was enriched in MPs [120].

PLA MPs and florfenicol were found to alter the ARG profile of the phytoplankton component *Chlorella pyrenoidosa* whose phycosphere hosts bacteria and ARGs and has a role in their spread in rivers. The exposure was carried out for 21 days in fish pond water and qPCR showed that the treatment with PLA MPs and florfenicol increased of more than two-fold the abundance of *sul*1, *sul*2, *flo*R, *fex*A, *fex*B, the oxazolidinone resistance gene *optr*A, *cfr* and the phenicol resistance gene *pex*A and MGEs *int*I1, IS613, *tnp*A01, *tnp*A02 and *tnp*A03 in the algal phycosphere that reached multiples of 10^{10} copies/L, and a relative abundance increase by 2 orders of magnitude. Metataxonomic and co-occurrence analyses revealed that in the phycosphere *Devosia*, *Flavobacterium*, *Hydrogenophaga* and *Methylophylus* were potential hosts for ARGs [122].

Samples of water, sediment, plastics, and fish intestine were collected in a *Sciaenops ocellatus* farm in Mauritius where fishes were grown in open cages and at different distances from a river estuary and a lagoon channel. Metataxonomic analysis highlighted that in floating macroplastic Proteobacteria abundance was higher than in water, sediments and fish gut with higher diversity of pathogenic microorganisms at the estuarine position and 30 bacterial species shared with the fish microbiota. Among these, *V. alginolyticus*, *Photobacterium damselae* and *Staphylococcus epidermidis* were isolated. Most Vibrionaceae showed high phenotypic resistance to ampicillin and tircacillin and about 3% were resistant to norfloxacin, ciprofloxacin, ofloxacin and levofloxacin but none to the antibiotics used in the farm. The MAR index was higher for plastic-associated bacteria [123].

In mariculture sediments MPs can reach a concentration of the order 10^5 particles per kg, at least ten-fold higher than in other sediments. PS and PVC particles with sizes ranging between 0–120 μm and 0.5–2.0 mm were incubated for 60 days in presence of tetracycline and sulfamethoxazole at the concentrations typically found in water and sediment collected in an aquaculture farm in Jiaozhou Bay (China). The microbiota present in sediments was pre-adapted to these antibiotics and the abundance of 13 ARG genes, determined by qPCR, increased to a maximum of 88% reaching the order of 10^8 copies/g. Among these, the *floR* gene known to be harbored by *Salmonella* spp. and previously reported in different seafood products. The greatest ARG profile distinctness was found between the PS and PVC particles. In PS *tet*W was selectively enriched, while in PVC the absolute abundance of *sul*1, *sul*2, and *sul*3 increased. These findings agree with the previously reported preferential adsorption of tetracycline in PS and sulfamethoxazole in PVC. ARGs *qac*H01 and *flo*R were significantly enriched in PS while *mat*A, *bla*TEM, *amp*276, *aad*A5, and *aad*E were significantly enriched in PVC. The relative abundance of *intI*1 and the transposase gene *tnp*A, increased in the presence of MPs and was positively correlated with the ARGs [29].

Metataxonomic analysis showed a significantly lower bacterial diversity in mariculture sediments exposed to PVC while exposure to PS enriched Proteobacteria, Bacteroidetes,

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Gemmatimonadetes, and Firmicutes. The neutral and null models showed that the PS treatment enhanced the migration of the microbiota components and ARGs to the sediment. Both polymers, but PVC at a higher extent, promoted the assembly of the microbiota by deterministic processes as under the effect of a selective pressure possibly exerted by the release of Zn, Cu, phthalates and BPA, and both polymers favored the increase of ARG hosts. Network correlation analysis indicated that PS enriched *Ralstonia*, *Planctomicrobium*, *Vulcanibacillus*, *Desulfitibacter*, and *Conexibacter* carrying *flo*R, *aad*A5, and *bla*$_{TEM}$, while PVC enriched *Tistlia*, Subgroup_10, *Candidatus_Alysiosphaera*, and *Pelagibius* carrying *tet*W, *sul*3, *mat*A, and *bla*$_{TEM}$. At the biofilm maturation stage both polymers were completely coated with bacteria and the 16S rRNA gene copy number reached multiples of 10^{12}/g but significantly lower for PVC and higher on smaller particles that are most likely ingested by benthic invertebrates such as mussels and clams [29].

In a high-density farm producing the shrimp *Penaeus vannamei* in the Guangdong coast (China) PE MPs of three sizes were deployed to investigate the presence of ARGs after two months. On the large particles level-I-risk ARGs including the genes for the *bla*$_{LCR-1}$, *cml*A5, the MLS resistance gene *lin*G, *aph(3'')-Ib*, *ant(6)-Ia*, *aph(6)-Id*, and *aac(6')-Ib9*, were significantly more abundant than in water. On MPs the dominant bacterial genera were different from those in water and the most abundant were *Paracoccus*, *Thauera*, *Thiobacillus*, *Halothiobacillus* and *Ruegeria*. The pathogenic genera *Brucella*, *Brevundimonas*, *Escherichia*, *Enterococcus*, and *Pseudomonas* were significantly more abundant than in water. The genera *Brucella* and *Brevundimonas* were positively correlated with *cat*B3 and *Pseudomonas* with *qac*H. On smaller MPs Sphingomonadales, Pirellulales, Desulfobulbaceae and Desulfocapsaceae and the genera *Vibrio*, *Kangsaoukella*, *Roseobacter*, *Roseovarius*, and *Shimia*, were enriched while Rickettsiales, and the genus *Granulosicoccus* were enriched on medium-size particles. A significant positive correlation was found between TOC and ARG abundance and a negative correlation was found between MP roughness and ARG abundance [114].

PBAT- and PP-MPs were deployed for three months in a *Tilapia* aquaculture farm in Haikou (China) in which 3,000 fishes were grown and antibiotics were used rarely and only in case of disease during the study period. The giant viruses *Pandoraviruses* that undergo gene duplications and are prone to HGT with their bacterial hosts prevailed on MPs. Chao1 index and the Abundance-based Coverage Estimator (ACE) were higher for PBAT than for other MPs and the biomass was higher for PHA and PLA. Bacterial assembly in MPs was driven by deterministic processes as shown by the Raup-Crick box plot compared to the other matrices analyzed, i.e. water, sediment and fish gut. Abundance and number of subtypes of ARGs where higher in MPs and *bac*A and *sul*2 were particularly abundant in PP. Moreover, MGEs were more abundant in PP and the *tnp*A transposase was more abundant in PBAT and PP. Rank I ARGs associated to the ESKAPE pathogens (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp.) and pathogens classified as Priority 1 (carbapenem-resistant *A. baumannii*, carbapenem-resistant *P. aeruginosa*, carbapenem and third-generation cephalosporin-resistant Enterobacteriaceae), Priority 2 (vancomycin-resistant *E. faecium*, methicillin and vancomycin-resistant *S. aureus*, clarithromycin-resistant *Helicobacter pylori*, fluoroquinolone-resistant *Campylobacter* spp, fluoroquinolone and third-generation cephalosporin-resistant *Neisseria gonorrhoeae*) [62] and Priority 3 fluoroquinolone-resistant *Shigella* spp. were found at higher levels in PBAT. Network correlation analysis showed stronger correlations between ARGs and microbial groups in MPs [124].

5.2. Selection of ARGs in Aquatic Animals Induced by MPs

An indirect mechanism of MP driven ARG selection and transmission in animal hosts is the induction of changes in the gut microbiota with the emergence of ARG carriers and activation of pathways favoring ARG spread. The zebrafish *D. rerio* is a model for studying the impact of different factors on the intestinal microbiota since it is simple to breed, has a short life cycle and structure and functional similarity of the intestine with the human gut. Oxytetracycline was combined with MPs and NPs to evaluate toxicity effects in this animal. After an exposure of 30 days, HT-qPCR with 384 primer sets targeting ARGs, MGEs and the 16S rRNA gene showed that the normalized copy

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numbers of ARGs and MGEs in the intestinal microbiota were significantly higher than the control in the treatments with NPs and MPs and NPs combined with oxytetracycline. The enriched ARGs in the treatments with MPs and NPs alone and with these combined with oxytetracycline were different with MDRGs more abundant for the MP and NP treatment. The microbiota composition was determined by metataxonomic analysis and Pseudomonadales and Burkholderiales were positively correlated with ARGs [125].

Exposure of the crucian carp *Carassius auratus*, a freshwater fish, separately or in combination to roxithromycin and PS MPs, not degraded or exposed to UV light with formation of hydroxyl groups and a modification of the carbonyl region in the Attenuated Total Reflectance FTIR (ATR-FTIR), determined an increase of the antibiotic in the fish intestine that was higher in the presence of MPs and even more for aged MPs. Moreover, inflammatory cell infiltration was observed in the intestine after exposure to the combined aged PS MPs and roxithromycin while cilia defects were observed after exposure to the antibiotic combined with not degraded PS MPs. Metagenome sequence analysis showed that the genera *Gemmobacter*, *Bosea*, *Rhizobium*, and *Shinella* increased in the intestinal microbiota after exposure to the MPs/antibiotic combination. Roxithromycin exposure led to an increased number of ARGs encoding resistance to bacitracin, tetracycline, sulfonamide and chloramphenicol and its combination with MPs led to the increase of *sul*1 positively correlated to different microbial genera [126].

In *C. auratus* exposed to aged MPs (AMPs) and roxithromycin for 14 days, the antibiotic accumulation in tissues and organs, especially gut, brain and kidney, was higher for smaller MPs. Compared with exposure to the antibiotic alone, the presence AMPs of 0.5 μm, 5 μm and 50 μm size led to an increase of *Proteobacteria, Fusobacteriota* and *Firmicutes* in fish gut and reduced the abundance of *Cetobacterium*, a probiotic genus that produces vitamin B12, butyrate and acetate, while *Shinella*, *Paracoccus*, *Gemmobacter*, *Pseudorhodobacter*, *Vibrio* and *Aeromonas* increased [127].

Oryzias melastigma or marine medaka euryhaline fishes were exposed for four weeks to PS MPs at about 10^8 particles/m^3 and tetracycline at 43 ng/mL, doses possibly found in the environment, singly or in combination. Metataxonomic analysis showed that exposure to tetracycline combined or not with MPs increased the levels of Proteobacteria and Bacteroidetes, lowered the levels of Firmicutes and Actinobacteria and changed the relative abundance of 15 bacterial genera in the intestinal microbiota. Among 35 tetracycline resistance genes sought by HT-qPCR, *tet*D, *tet*G-02, *tet*O, *tet*Q, *tet*R, *tet*W, *tet*35 and *tet*34, were detected in the group exposed only to the MPs, and *tet*O, *tet*R, *tet*W and *tet*35, in the tetracycline/MP combination group. Therefore, it was concluded that the increase in the number of the *tet* ARGs in the group exposed to PS MPs alone was a consequence of their effect on the intestinal microbiota [128].

Biofilms in MPs with adsorbed oxytetracycline were artificially obtained and tested for their effects on zebrafish after 30 days of exposure in comparison with biofilms on MPs without adsorbed oxytetracycline, MPs alone, and the control. Metagenome sequence analysis showed that the relative abundance of intestinal *Proteobacteria* increased after exposure to MPs with biofilm with or without oxytetracycline and *Fusobacteria* increased mostly after exposure to MPs with biofilm and adsorbed oxytetracycline. The number of ARG subtypes significantly increased in all MP treatments, with or without biofilm and it was 104 for the group exposed to MPs with biofilm, and 101 for the group exposed to MPs with biofilm and oxytetracycline. MDRGs were the most numerous resistance genes in each group, followed by ARGs for macrolides, vancomycin and tetracyclines in this order. Moreover, a strong correlation was found between pathogenic bacteria and ARGs [129].

Exposure for 8 weeks to PVC MPs combined with either sulfamethazine or oxytetracycline exerted no significant effects on body weight and length of carps *Cyprinus carpio* but metataxonomic analysis revealed effects on the intestinal microbiota composition. The groups exposed separately to PVC, sulfamethazine, oxytetracycline and to PVC/sulfamethazine showed higher diversity of the gut microbiota than the group exposed to PVC/oxytetracycline and the group exposed to PVC/sulfamethazine showed the highest alpha-diversity. Exposure only to PVC or the antibiotics induced an increase of *Fusobacterium* spp., and Firmicutes that was enhanced in the

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PVC/oxytetracycline group. The genus Enterobacter, host of the MDRGs *acr*A-05, *acr*R-01, *tol*C-02, and *tol*C-03 and tetracycline ARGs *tet*D-01, *tet*D-02, and *tet*R-02, was more abundant in the groups exposed separately to PVC and sulfamethazine. HT-qPCR showed that 101 ARGs and 8 MGEs were present in all groups but in higher number in the single antibiotic and the PVC/sulfamethazine group. Treatment with PVC/oxytetracycline enriched tetracycline resistance genes, mostly *tet*M-01, *tet*M-02 and *tet*PB-03, while the PVC/sulfamethazine treatment led to the increase of *cmxA*. Most ARGs were associated to Proteobacteria and Actinobacteria and the composition of the microbial population had a greater influence on ARG distribution than on MGE occurrence [130].

ARG HGT in the mussel *M. galloprovincialis* was investigates upon exposure to PE MPs covered by a biofilm formed in a seawater simulating solution by *E. faecium* UC7251 and *L. monocytogenes* strains Scott A and DSM 15675. The *E. faecium* strain harboring *ant(6)-Ia*, *aph(3')-III*, *aad(6)-Ia*, *ant1*, *erm*B, *mrs*C, *sat*A, *isa*E, *lnu*B, and *tet*L in an integrative and conjugative element (ICE) present on a mobilizable megaplasmid and the transposon Tn916 carrying *tet*M on the chromosome was used as MDR ARG donor and *L. monocytogenes* strains were used as pathogenic recipient representatives. Conjugation with HGT of *tet*M and *erm*B did not occur between planktonic cells but the transfer of *tet*M occurred in the MP biofilm and in mussels exposed to the MPs at a higher extent than in water so it was concluded that the MP concentration consequent to the filtering activity of mussels favored ARG HGT [131].

The widely used antibiotic ciprofloxacin was detected in different aquatic environments at concentrations ranging from a few to hundreds μg/L and between 9.1 and 14.54 μg/kg in Asian green mussel *Perna viridis* farmed on the Chinese coastline. In shellfish aquaculture MP presence is caused mainly by usage of plastic structures, such as rope cultivation supports. The intestine of *P. viridis* can accumulate MPs at average levels of 10 particles per mussel for a surface area of about 3 m^2/g, pores of about 20 μm and prevailing PP composition. Exposure to artificial MP particles at a concentration of 0.6 mg/L and to 1 μg/L ciprofloxacin mimicking the amounts found in the environment led to a 60% higher concentration of the antibiotic in mussels compared to exposure to ciprofloxacin alone with an average final concentration of 142 μg/kg wet weight. Metagenomic analysis highlighted a decrease of the genus *Spirochaeta* and lactic acid bacteria and an increase of the pathogenic genera *Treponema* and *Vibrio* in the mussels exposed to the antibiotic. Rather than a change in the types of ARGs, a shift in ARG carriers was observed as an effect of selective pressure, for example MLS ARG carriers shifted from Paenibacillaceae to Lachnospiraceae. The target hazard quotients (THQs) and cancer risk (CR) indicated a negligible toxic risk associated with frequent or occasional consumption of the contaminated mussels by children and adults but these risks were significantly heightened. The mussels exposed to MP and cyprofloxacin after cooking exceeded the lower value of Minimal Selective Concentration (MSC) of the antibiotic, namely a concentration lower than the MIC, that can select for resistant bacteria as dietary exposure dose for the human gut microbiome (DEGM) [132].

The enhancement of veterinary antibiotics oxytetracycline and florfenicol accumulation in edible bivalve molluscs upon exposure to MPs was observed and was explained with the suppression of detoxifixation genes. The level of accumulation was well below the the estimated THQs but the DEGMs for the antibiotics tested were greater than or similar to the MSC thus indicating a risk for AMR development [133].

Macrobenthic invertebrates such as shrimp, shellfish and snails retain plastic particles, mostly NPs, from the surrounding water at a rate proportional to their biomass and different species of aquatic invertebrates were found to carry ARGs. In spiral shells at different sites it was observed that NPs were only found in the animal gut while bigger particles were present in significantly lower amounts than in the sediment. PET, PP, PU, PVC, PA and PS MPs were found in comparable concentrations in the shellfish gut and in the sediment. All the benthic macroinvertebrates, and particularly *Chironomidae* larvae, showed higher MP concentrations than water and benthic fish gut. Among ARGs specifically detected by qPCR, *tet*A, *sul*1, *sul*2, and *sul*3, were always at higher levels in spiral shells and the gene *tet*A showed a higher ratio between copy number and number of plastic particles and a negative correlation between the copy number and the particle size. To elucidate the

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effect of MP degradation on the increase of ARG copy number, *Chironomidae* larvae were fed with PE MPs of about 42 µm and *E. coli* K12 MG1655 harboring the conjugative plasmid RP4. The number of MP particles and their size decreased during the experiment as a consequence of degradation. The copy number of the plasmid gene *traG* determined by qPCR increased in presence of MPs, in particular when the *E. coli* strain was fed after 15 days of MP exposure thus indicating enhanced conjugative transfer after MP degradation. Moreover, the conjugative genes *trfAp* and *trbBp*, and the SOS response genes *recA*, *polB*, *ruvA*, *recN*, and *uvrB* were strongly upregulated in the gut of the *Chironomidae* larvae upon exposure to MPs and *E. coli*, more than upon exposure to *E. coli* alone, particularly after 15 days of MP feeding. In a *E. coli deletion* mutant for the *lexA repressor* the HGT of plasmid RP4 was more efficient than in the native strain confirming the involvement of the SOS response [134].

An in situ exposure experiment of *M. coruscus* to PE MPs was conducted on the Xixuan Island (China) for 21-days to evaluate the effect on the viral community composition in the digestive gland. Shotgun metagenomic showed that 25 viral operational taxonomic units (vOTUs) in the MP microbial biofilm carried ARGs predominantly associated with MDR and MLS resistance for a total of 22 subtypes. The relative abundance of ARGs in the digestive gland of *M. coruscus* after MP ingestion significantly decreased except for MDR genes such as *efr*A and *pat*B which increased [135].

PE and PET MPs aged for three months in a flow-through oyster *Magallana angulata* aquaculture farm harbored a number of copies of the 16S rRNA gene of the order of 10^{10}/g and a number of copies of *intI*1, *sul*1, *sul*2, *tet*C, and the plasmid-encoded multi-resistance efflux pump *oqx*B at copy numbers of 10^6/10^7, 10^8, 10^7, 10^6 and 10^5/g, respectively. The gene *tet*G was present in about 10^7 copies/g on PET and the number of copies of *intI*1, *sul*1, *erm*B, and the chloramphenicol exporter *fex*A significantly differed between the two plastic types. In the oysters exposed to PE for 14 days the genes *oqx*B and *tet*A increased by about 8 and 12-fold, while in those exposed to PET *oqx*B, *tet*G and *intI*1 increased by 6, 5 and 1- fold, respectively. The number of copies of *sul*1 was significantly higher in the PET group compared to the PE group. In oyster excreta all ARGs were enriched, except for *tet*C, indicating that the excreta can transmit the ARGs to other aquatic organisms with consequent introduction in the food chain. The *intI*1 gene increased in oysters exposed to MPs and their excreta, and in oysters it was positively correlated with *sul*2 and *tet*G while in the excreta it was positively correlated with *sul*1 [136].

One study analyzed the effect of trophic transfer of MPs on ARG selection in aquatic animals examining the effect of PP-MPs in the transfer of oxytetracycline from the shrimp *Neocaridina denticulata* to the crucian carp *Carassius carassius*. Shrimps were exposed to 200 µg/L oxytetracycline, a concentration in the range of those found in aquaculture environments, alone or in association with 100 µg/L PP-MPs, and fed to carps in controlled conditions. After 14 days of exposure, oxytetracycline levels in shrimp gut and carp liver and gut was significantly higher in presence of the MPs/antibiotic combination compared to the antibiotic alone. Oxytetracycline induced an increase of Actinobacteria and Firmicutes in the shrimp, and Bacteroidetes in the carp, while its combination with MPs it induced the increase of Actinobacteria in the shrimp and Firmicutes in the carp. Exposure to oxytetracycline and MPs from water increased the number of *Gemmobacter*, *Rhizobium*, and *Shinella* and pathogenic bacteria in both animals compared to the antibiotic alone, while trophic assumption of oxytetracycline and MPs by the fish led to an increase of the genera *Gemmobacter*, *Pseudorhodobacter*, *Cetobacterium* and *Acinetobacter*, and a decrease of *Akkermansia* and *Bacteroides* to a greater extent than when assumed directly from water. MPs enhanced the increase of *tet*A, *cml_e3*, *sul1*, *tet*G, and *aph33i*B in the shrimp gut induced by oxytetracycline and *tet*E, *tet*A, *sul1*, and *tet*M increased in the carp gut after exposure to MPs and oxytetracycline through water while only *tet*E increased after exposure to the sole oxytetracycline. This increase was enhanced when the carp assumed the antibiotic by eating the shrimp exposed to oxytetracycline and even more when the shrimp was also exposed to MPs. In this case, *tet*A and streptomycin ARGs increased suggesting that MPs adsorbed oxytetracycline enhancing its trophic transfer. Based on correlations networks, the ARGs *tet*A, *tet*G, *sul*1, *catb3*, and *cml_e3*, *aph33i*B and *aph6i*D had a higher number of potential hosts in the shrimp intestine while *tet*M,

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*tet*B, *tet36*, *tet*C and *aac6i*B had a higher number of potential hosts in the intestines of the crucian carp comprising *Clostridium*, *unclassified_f_* Clostridiaceae and *Bacteroides* [137].

According to the studies reviewed here, some connections were established between exposure to MPs associated or not with antibiotics and ARG selection in aquatic animals. The most remarkable are illustrated in Figure 1.

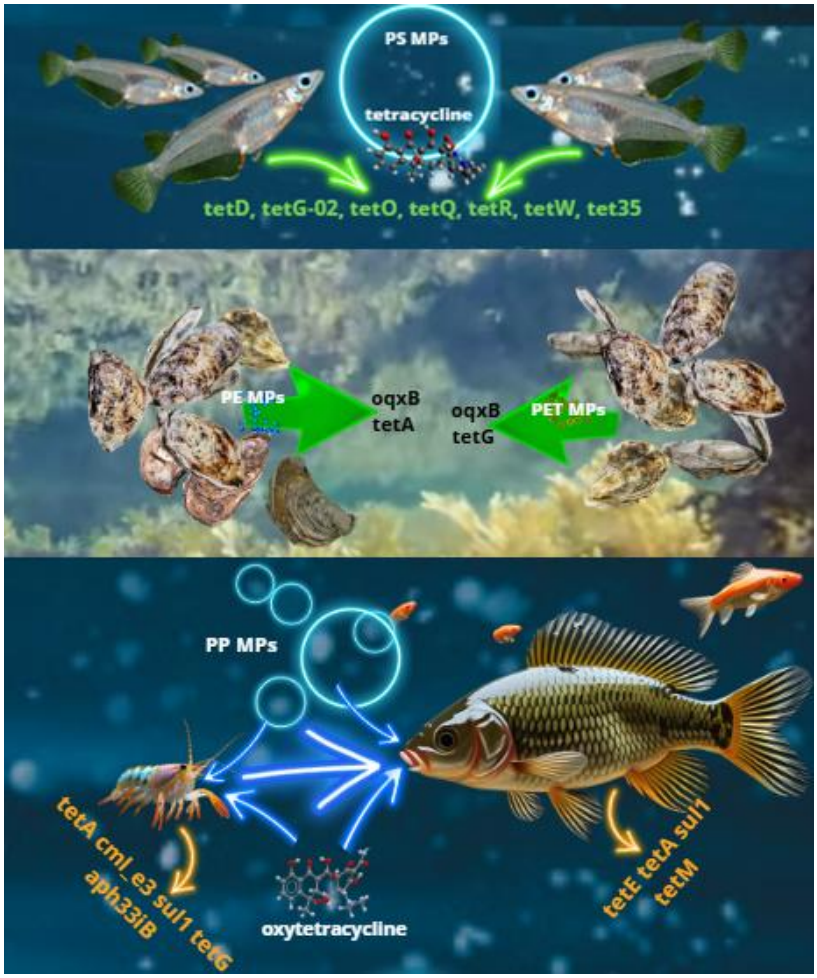

Figure 1. The demonstrated effects of exposure to MPs and antibiotics on ARG enrichment in fishery products. PE (polyethilene), PET (polyethylene therephtalate), PP (polypropylene) [128,136,137].

5. Discussion

This comprehensive review collected the evidences from numerous recent studies that plastic wastes of different dimensions favor the selection and the enrichment of ARB and ARGs in water environments. This mainly occurs because MPs function as a solid support for the formation of a bacterial biofilm that favors HGT events involving MGEs whose abundance was often found to be positively correlated with that of ARGs and MDRGs and/or enriched in the plastisphere [29,40,44–47,49,50,60,64,67,69,73,74,76–78,82–84,89,90,96–99,103–108,117,118,120,122,124,136]. Even if some studies reported an ARG abundance in plastic biofilms lower than in water, still positive correlations of ARGs with MGEs and bacterial carriers were found in the plastisphere thus indicating an increased HGT risk in MPs [40,44,46]. In particular, the occurrence of ARGs in MPs lower than in water found in those few studies contradicts the findings of the other investigations but the inconsistency is most probably due to the use of targeted detection of relatively few ARGs by qPCR that might have missed other ARGs present.

The studies affording the MP route of AMR transmission increased yearly from 1 and 2 in 2018 and 2019, respectively, to 5 and 7 in 2020 and 2021, respectively, 14 in 2022 and 2023 and 24 in 2024.

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In the current year 21 studies were published, indicating the increased interest of the scientific community for the concern posed by plastic wastes in AMR spread in water environments in which a fauna including edible species is directly exposed to these contaminants.

Though only eight studies analyzed the effect of exposure to MPs/NPs and antibiotics on the AMR profile of aquatic animals, the ARG selection by MPs in the gut microbiota of fishes, crustaceans and mollusks was demonstrated [125,127–129,131,132,136,137]. Moreover, the increase of ARGs and MDRGs in aquatic organisms also by NPs was demonstrated in *D. rerio* [125]. Therefore, the widespread presence of ARGs and MDRGs in plastic debris in aquatic environments such as rivers, lakes, estuaries, mangroves, sea, coral reefs and aquaculture farms [29,39–47,49–67,69,70,73–108,116–137] are indicative of undesired effects occurring in aquatic animals and of the need to reduce their exposure to MPs to limit the risk of ARG introduction in the food chain.

ARG selection and HGT in the plastisphere occur in waterbodies with different degrees of salinity and was reported in different countries, even in extreme climates [43,94] showing that plastic wastes are a hotspot of AMR spread globally. The majority of in situ and ex situ studies on ARGs and MDRGs presence in the plastisphere in aquatic environments was carried out in China and regarded both waterbodies and aquaculture farms [29,40,44–50,53,56,57,59,64–69,82,83,92,96–99,101–103,105,108,114,116–118,120] while rather few studies were carried out in Europe [42,43,51,54,73,75–79,85,86,88,90], four in Asian countries other than China [61,89,104,107], four in African countries [81,91,93,125], one in Antarctica [94] and one in America [100]. This uneven study distribution implies that a preponderant part of the globe is still unmonitored and the issue of ARG enrichment by plastic wastes in waterbodies is still not contrasted compared to other measures adopted to contain the AMR pandemic [138].

Nevertheless, the problem of MP presence and plastisphere development in rivers, lakes and seas as final acceptors of wastewaters is of great concern since MPs, owing to their low density, remain suspended in waters and are transferred from WWTPs, agricultural and industrial runoffs to waterbodies with their microbial load [59,69,72]. Some of the studies clearly showed the relevant role of WWTPs in reshaping the composition of microbial consortia and ARG assembly in MP biofilms [45,72,73,76,78,79,81,88]. On the other hand, it was reported that the MPs can be covered by a biofilm even in tap water where they could promote ARG enrichment and spread [76]. Moreover, plastic aging due to exposure to light and fragmentation enhances the bacterial adhesion rate and ARG enrichment [15,126,134].

Some specific ARGs and MDRGs were detected in multiple studies and most of these in all aquatic environments considered in this review, i.e. freshwater, seawater, estuarine sites and aquaculture farms. Those detected in all four environments were *sul*1 reported in 31 instances [29,42,45,48,50,51,53,58–60,66,75,76,78,80,81,83,88,89,92,96,99,101,103,115,117,120,122,126,134,136,137], *sul*2 in 21 instances [29,39,50,53,58,60,66,69,78,81,86,92,96,97,101,115,117,118,122,124,134,136], *tet*A in 17 instances [39,45,48,54,58,74,81,84,86,89,96,97,99,101,134,136,137], *bla*$_{TEM}$, *erm*B and *tet*W in nine instances [29,40,42–45,48,58,61,69,71,81,86,88,89,93,99,101,120,128,136] and *erm*F in eight instances [44,60,79,81,96,97,103,117]. MDRGs were detected in 25 studies and in most cases more than one representative was detected in the same environment so it can be stated that MDR was more widespread than resistance to single antibiotic classes [38,45,47,49,53,56,58,66,67,78,81–84,87,90,98,102,104–106,119,125,129,130]. ARGs not found in all the environments but detected both in freshwater and seawater showing to be little affected by salinity were *bac*A reported in 14 studies [54,56,64,66,67,81,83,84,90,98,100,104,105,123], *tet*C in 11 studies [39,48,50,51,66,71,74,84,89,136,137], *qnr*S in eight studies [39,59,77,86,89,90,117,120], *tol*C in six studies [60,64,84,90,104,130] and *qnr*A in four studies [39,59,85,117]. Other genes were detected in freshwater and in aquaculture farms and some were also present in estuarine environments. These, ordered in decreasing number of reports from eight to five, were, *tet*G, *dfr*A1, *ere*A, *tet*B and *tet*M [40,41,45,49,50,52,58,59,64,69,74,78,81,83,89,101,117,118,128,130,136,137]. Other genes present both in freshwater and seawater and some detected also in estuarine waters but not in aquaculture farms

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were the efflux pump gene *acr*B in seven studies [54,58,66,81,84,90,104], *mac*AB, *mex*F and *ceo*B in four studies and the MDR transporters *msb*A, *ros*A, and *fos*X in three studies [47,49,53,56,57,66,67,81–84,96,97,100]. As MGE indicator, the integrase gene *intI*1 was most frequently detected in all the environments, as reported by 29 studies [29,40,44,48,50,51,53,54,58–60,64,66,71,73,74,76,78,79,89,96,97,99,103,105,117–120,122,136]. The next most frequently detected MGE indicator in both freshwater and seawater was *tnp*A [29,53,57,66,78,83,124] with its variants found only in freshwater [39,45,47,50,53,57,59,60,66,83,122].

Notably, different studies reported the detection of genes for ESBLs, ARGs of major global burden [139] in plastic biofilms [29,41,47,48,51,58,59,61,77,78,83,84,86,88,93,101,104,114,118,120] and the ability of bacteria isolated from the plastisphere to disseminate ESBL via conjugation was demonstrated [56,77].

Compared to the systematic review of Zhu et al. [38] in which only metagenome datasets were considered with a predominance of those from PVC, in this literature survey also results obtained with other molecular techniques and experimental approaches were considered. Results agreed on the higher ARG abundance in BDPs compared to NBPs but not in the ranking of the NBPs based on ARG abundance. The results on the frequency of ARG types detected agreed in the predominance of MDRGs, sulfonamide and tetracycline ARGs. MDRGs presence was reported by 25 studies reviewed here and most found the co-occurrence of several of those genes [38,45,47,49,53,56,58,66,67,78,81–84,87,90,98,102,104–106,119,125,129,130]. Aminoglycoside ARGs that were indicated as the most abundant after tetracycline ARGs by Zhu et al. [38] and predominating in the study of Yang et al. [84], were reported by 23 studies commented here and were represented by a diverse group of genes [29,41,60,66,67,69,78,81,83–85,104,105,114,137].

According to some studies, the type of plastic polymer influenced transformation and conjugation frequency and the selection or abundance of different bacterial groups, ARG classes and MGEs [22,27–29,33,38,39,42,43,45,48–50,52–54,56–60,64,67,69,75,78,79,89,94,96,98,100,103,106,107,120,124,136]. PP was identified in different studies as the polymer with highest tendency to promote biofilm formation. In particular, it showed a higher biomass abundance compared to PE and PS in a study of mixed biofilm formation and HGT between *B. subtilis* and *A. baylyi* [22]. Moreover, the MCDM method VIKOR indicated PP as the material with the highest risk of HGT since biomolecule interaction prediction indicated that PP stress strengthened the binding of active codons by the relaxase and VirB5 proteins involved in conjugation [28]. The ARG assembly in PP in freshwater was found to be dominated by stochastic processes [57] and ciprofloxacin-resistant bacteria were more abundant in PP upstream and downstream from a WWTP in the Mondego River (Portugal) [77,78,96]. In addition, PP, followed by PE was the polymer harboring more viral ARGs and positively correlated with the highly positive z potential that favor the binding of negatively charged DNA [107]. Network correlation showed more numerous interactions between viruses and bacteria on PP [107] and PP also showed the highest antibiotic adsorption rates in mariculture [116]. Based on the above findings, it appears that PP binds more efficiently than other NBPs microorganisms, environmental DNA and antibiotics, thus representing a high-risk material for ARG HGT and spread.

Several studies indicated PVC as the polymer with highest tendency to release toxic components. In particular, bacterial diversity was lower than on other polymers deployed in the Henares River (Spain) for the possible release of inhibitory substances [79] and also in mariculture sediments exposed to PVC a significantly lower bacterial diversity was observed. Moreover, it was shown that PVC more than other polymers promoted a microbiota assembly driven by deterministic processes that could depend on a selective pressure possibly exerted by the release of Zn, Cu, phthalates and BPA [29]. On the other hand, it was demonstrated that the PVC leachate promoted ARG increase [87] and HGT with a synergistic effect with PVC particles in a study in which use of green fluorescing transconjugants allowed to observe their appearance on the surface of PVC earlier than on PS and with a higher ratio of transconjugants to donors. The conjugal transfer on PVC was

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possibly favored by oxidative stress as inferred from the higher abundance of ROS in bacteria adhering to PVC [29].

BDPs, among which PLA is the most largely used and whose amount is increasing globally, also cause ARB and ARG enrichment favored by the high tendency to fragmentation with the formation of small particles. Different studies showed that BDPs can pose a higher risk than NBPs in ARG increase and spread [49,50,57,59,64,71]. Moreover, a synergistic effect with the toxic plasticizer DBP was demonstrated for PLA [32].

Part of the in situ studies commented here established vague relationships between bacterial hosts and ARGs or MDRGs while other associated bacterial groups and, in some cases, definite bacterial genera or species to the detected ARGs by network correlation analysis or experimentally [Tables 1–3]. Moreover, predictive models were developed to associate specific ARGs to the respective bacterial hosts [38]. Establishing these connections precisely could allow to limit the probability of ARG HGT in the plastisphere by reducing or preventing contamination by particular microbial groups able to transmit or acquire AMR genes. Indeed, the sources of these microorganisms could be identified and more strictly and efficiently controlled. Since, different studies demonstrated the contribution of WWTP effluents and urbanization to ARG enrichment in the plastisphere, the improvement of the water purification process is pivotal in the mitigation of the related risks. Therefore, further efforts should be dedicated to the study of the effects of wastewater treatments in reducing the persistence of specific ARB [69]. In particular, action is required in improving the effectiveness of constructed wetlands in eliminating ARB, ARGs and MPs that are implicated in their selection [140].

Another source of ARG harboring MPs are the aquaculture plants and the task of simultaneously eliminating MPs and ARB/ARGs from aquaculture effluents is challenging. Different chemical, physical and biological technologies and their combinations were tested but still need optimization. Feasible treatments are MP physical removal by coagulation and filtration through materials such as polyaluminum chloride, magnetic biochar modified with quaternary phosphonium salt and reverse osmosis, and ARG/MGE elimination through chemical oxidation by Fenton or H_2O_2 oxidation, ozone or polysulfate treatment, or anaerobic combined oxidation. Promising technologies are advanced oxidation processes with a combination of UV light and H_2O_2, or high-frequency electromagnetic field with low-dose chlorine [141].

A biological purification method exploits the symbiosis between bacteria and algae in constructed wetlands (CWs), that has the potential to remove both MPs and ARGs. In this consortium algae utilize CO_2 to produce organic matter that can be transformed by bacteria into inorganic matter utilized by the microalgae. These generate large amounts of oxygen by photosynthesis and that facilitates the degradation activities of aerobic bacteria. Use of suspended algae such as *Scenedesmus* and *Chlorella* was found to quickly remove high amounts of MPs and ARGs from aquaculture water. However, at this time research on the removal of MPs by bacteria and algae consortia is still limited. Another promising strategy exploiting a biological process is based on the microbial fuel cells (MFCs) in which microorganisms able to produce electricity and antibiotic-degrading bacteria form a biofilm on the anode. In this system antibiotics are degraded while electricity is generated thus allowing aquaculture wastewater decontamination with production of bioelectric energy. The efficiency of the mentioned decontamination processes is strongly influenced by environmental factors so they still require optimization [141].

Another aspect to be further explored is the role of MPs in increasing the ARG content in natural matrices such as water and sediment. Indeed, a recent study based on long read metagenome analysis of river water and sediment microcosms showed that the presence of MPs increased the diversity of ARGs in the surroundig matrices. Indeed, in presence of MPs after 7 and 14 days a higher number of ARGs were detected in the sediment, among which *bla*$_{\text{TEM-116}}$, the oxytetracycline resistance gene *otr*C, the MLS efflux pump gene *ole*C, and *oqx*B at day 7 and *dfr*B3, the tetracycline efflux pump *tcr*3, *ole*C, and *otr*A at day 14. Moreover, MPs determined the increase of *Aeromonas* spp. i.e. *A. salmonicida*, *A. hydrophila* and *A. veronii*. For water samples changes in the relative abundance of Proteobacteria

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classes were observed and ARGs *dfr*B3, *mph*E, *ole*C, *tcr*3 and *otr*A were detected at day 7 and *qep*A4, *oqx*B and *otr*A at day 14 while in absence of MPs only the *vat*F gene responsible for resistance to dalfopristin, pristinamycin, and virginiamycin was detected [142].

6. Conclusions

The concern of ARBs and ARGs selection and increase in waterbodies favored by plastic wastes was observed in most of the studies affording this subject and all agreed on the facilitation of HGT in the plastic biofilm for the enrichment in MGEs and their positive correlations with ARGs and/or their bacterial hosts. As a consequence, the directly exposed aquatic animals beyond suffering the direct toxicity of plastic particles such as MPs and NPs are also affected by the perturbation of the gut microbiota with selection of ARG carriers. These can be propagated by trophic transfer and can be particular detrimental for human health if present in edible species, particularly those that are consumed raw. New studies linking plastic contaminants with ARB and ARG selection should be aimed at elucidating the factors that favor ARB establishment and ARG propagation in aquatic environments to find strategies that can mitigate the associated risks. The number of studies aimed to evaluate the effects of MPs covered by a plastisphere on ARG selection in aquatic organisms and their transmission in the food chain should be increased to better estimate the dimensions of the problem.

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List of Abbreviations

ACE	Abundance-based Coverage Estimator
ADI	Acceptable daily intake
AHL	Acyl-homoserine lactones
AMR	Antimicrobial resistance
APS	Artificial plastic substrate
ARB	Antibiotic-resistant bacteria
ARG	Antibiotic resistance gene
ARISA	Automated ribosomal intergenic spacer analysis
BDP	Biodegradable plastic
BPA	Bisphenol A
CF	Cellophane
CR	Cancer risk
DBP	Dibutyl phthalate
DCFH-DA	2′, 7′ - Dichlorofluorescein diacetate
DEGM	Dietary exposure dose for the human gut microbiome
DEHP	(2-ethylhexyl) Phthalate
EDI	Estimated daily intake
EPS	Extracellular polysaccharides
ESBL	Extended spectrum beta-lactamase
ESKAPE	*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Enterobacter* spp.
FTIR	Fourier transform infrared
GBDT	Gradient boosting decision tree
GHRI	Genotypic health risk index
HDPE	High-density PE
HGT	Horizontal gene transfer

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HQ	Human health risk quotient
HT-qPCR	High throughput qPCR
HTS	High throughput sequencing
ICE	Integrative conjugative element
IHRI	Integrated health risk index
LDPE	Low-density PE
LGBM	Light Gradient Boosting machine
LSCM	Laser scanning confocal microscopy
MAG	Metagenome assembled genome
MALDI	Matrix-assisted laser desorption ionization
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization Time-of-Flight
MAR	Multiple antibiotic resistance
MARB	Multiresistant bacteria
MCDM	Multiple criteria decision making
MD	Molecular dynamics
MDR	Multidrug resistance
MDRG	Multidrug resistance gene
MGE	Mobile genetic element
MIC	Minimum inhibitory concentration
ML	Machine learning
MLP	Multilayer perceptron
MLR	Multiple linear regression
MLS	Macrolide-lincosamide-streptogramin
MP	Microplastic
MRSA	Methicillin-resistant *S aureus*
MSC	Minimal Selective Concentration
NBP	Non-biodegradable plastic
NCM	Neutral community model
NP	Nanoplastic
OTU	Operational taxonomic unit
PA	Polyamide
PAH	Polycyclic aromatic hydrocarbon
PBAT	Poly-butyleneadipate-co-terephthalate
PBD	Polybutadiene
PBS	Polybutylene succinate
PCB	Polychlorinated biphenyl
PCL	Polycaprolactone
PCoA	Principal coordinate analysis
PDMS	Polydimethylsiloxane
PE	Polyethylene
PET	Polyethylene therephthalate
PF	Phenol formaldehyde
PF	PE-fiber
PFP	Pentafluorophenyl acrylate
PFP	PE-fiber-PE
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PLA	Poly lactic acid
PLC	Polyisoprene chlorinated
PMMA	polymethyl methacrylate
PMMA	Polymethyl methacrylate
PP	Polypropylene
ppLFER	Poly-parameter Linear Free-Energy Relationships
PPR	Projection pursuit regression
PS	Polystyrene
PTDL	Polytridecanolactone
PU	Polyurethane
PVC	Polyvinyl chloride
QS	Quorum sensing
QSPR	Quantitative Structure Property Relationship

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RF	Random forest
RM	Random forest
RMSD	Root-mean square deviation
RMSE	Root Mean Square Error
ROS	Reactive oxygen species
SAN	Styrene acrylonitrile resin
SC	Specific conductivity
SEM	Scanning electron microscopy
SHAP	SHapley Additive exPlanation
SRA	Sequence Read Archive
SSA	Specific surface area
T4SS	Type IV secretion system
TAOC	Total antioxidant capacity
TDS	Total dissolved solids
THQ	Target hazard quotients
TOC	Total organic carbon
TWP	Tire wear particles
vOTU	Viral operational taxonomic unit
WGS	Whole genome sequencing
W-LDPE	Waste LDPE
WWTP	Wastewater treatment plant
XGB	eXtreme Gradient Boosting

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