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Microplastic-Associated Biofilm Formation and Its Role in Harbouring Foodborne Pathogens in Aquatic Food Sources

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Abstract- ***Microplastics are emerging vectors for microbial colonization in aquatic ecosystems, with potential implications for food safety. This study investigated microbial composition, biofilm characteristics, and pathogen survival on microplastics, as well as their capacity to transfer foodborne pathogens to aquatic organisms. Microplastic samples (PE, PP, PS, PET) were collected from rivers, lakes, and coastal waters using plankton nets and manual sampling. In the laboratory, biofilms were recovered from microplastic surfaces via vortexing and sonication, followed by microbial enumeration on selective and non-selective media. DNA was extracted for 16S rRNA gene sequencing and analyzed using QIIME2 to determine bacterial composition and identify potential pathogens. Fluorescence microscopy and confocal laser scanning microscopy (CLSM) assessed biofilm structure, thickness, and viability. Survival of pathogens (E. coli, Salmonella spp., Staphylococcus aureus, Listeria monocytogenes) was evaluated under varying temperatures (15–35 °C), salinity (0.5–30‰), and nutrient levels (0.1–1% glucose) over 7 days. Pathogen transfer experiments involved exposing tilapia (Oreochromis niloticus), shrimp (Litopenaeus vannamei), and mussels (Mytilus edulis) to microplastics under controlled aquaculture conditions, with CFU enumeration, qPCR, and microscopy confirming colonization. Results showed that microplastics harbored diverse microbial communities dominated by Proteobacteria, Bacteroidetes, and Firmicutes, with several potential pathogens present. Larger and fibrous particles supported higher pathogen loads, and environmental conditions influenced microbial persistence. Exposure experiments confirmed that microplastics facilitate pathogen transfer to aquatic organisms, particularly in mucus-rich tissues. These findings highlight microplastics as reservoirs and vectors of foodborne pathogens, posing ecological and public health risks. Effective monitoring and mitigation strategies are needed to reduce microplastic-mediated pathogen transfer in aquatic environments.***

Index Terms- ***Microplastics, Associated, Biofilm Formation, Harbouring, Foodborne Pathogens.***

I. INTRODUCTION

Microplastics, defined as plastic particles smaller than 5 millimeters, have become ubiquitous pollutants in aquatic ecosystems. Their small size, high surface area, and chemical properties make them ideal substrates for microbial colonization, leading to the formation of biofilms. These biofilms are structured microbial communities embedded in extracellular polymeric substances, which protect microorganisms from environmental stress and facilitate their survival. The colonization of microplastics by microbes alter aquatic microbial ecology and have important implications for human health, particularly through the contamination of aquatic food sources. A major concern is that microplastic-associated biofilms can harbor pathogenic microorganisms, including bacteria responsible for foodborne illnesses (Lund et al., 2024). Aquatic food sources such as fish, shellfish, and other seafood are exposed to these microplastic particles, which can serve as carriers of harmful microbes. The biofilm structure enhances the persistence and resistance of pathogens to environmental stressors, increasing the likelihood of contamination during harvesting, processing, and consumption. In this way, microplastic-associated biofilms may act as reservoiurs and vectors for foodborne pathogens, representing a potential risk to public health. The mechanisms that drive biofilm formation on microplastics and the interactions between pathogens and these surfaces are influenced

by multiple factors, including the physicochemical properties of the plastics, nutrient availability, temperature, and salinity. These factors can promote microbial adhesion, growth, and survival, enhancing the stability and virulence of pathogenic organisms. Understanding these interactions is crucial for assessing the risks posed by microplastics in aquatic food systems and for developing effective strategies to minimize microbial contamination (Cholewińska., 2024).

Zhang et al. (2025) investigated the microbial community composition of biofilms on microplastics in coastal waters, using high-throughput 16S rRNA sequencing, and found that pathogenic bacteria such as *Vibrio* and *Aeromonas* were enriched on microplastic surfaces, constituting approximately 12–15% of total bacterial abundance compared to <2% in surrounding water. Çelik et al. (2025) characterized seasonal variations in microplastic-associated biofilms in a river system by collecting PE and PP particles across seasons and applying metagenomic analysis, reporting that *Salmonella* abundance increased from 5% in winter to 18% in summer on microplastic surfaces. Lo et al. (2025) examined colonization dynamics in an aquaculture pond using in situ biofilm sampling and microbial culturing, revealing *Listeria monocytogenes* counts of 1.2 × 10^4 CFU/cm^2 on PE microplastics after 30 days of incubation. Rahman et al. (2025) assessed pathogen distribution on microplastic biofilms in estuarine waters via qPCR and found that *E. coli O157:H7* and *Campylobacter* spp. reached concentrations of 3.5 × 10^3 gene copies per particle. Reisoglu et al. (2024) compared microbial communities on natural substrates versus microplastics using 16S/18S rRNA sequencing, reporting that potential pathogens were 6–8 times more abundant on plastic surfaces than on stones or sediments. Lund et al. (2024) investigated wastewater treatment plant effluent microplastics using culture-based detection and sequencing, documenting multidrug-resistant *Enterobacteriaceae* at 2.1 × 10^3 CFU/L on MP surfaces post-treatment. Tang et al. (2024) explored biofilm development on PE, PVC, and PP in aquaculture tanks using microscopy and community profiling, finding that PE biofilms had a biomass of 1.5 mg/cm^2 and harbored 1.8 × 10^4 CFU/cm^2 of pathogenic bacteria. Gross (2025) tested particle size effects on pathogen adhesion in laboratory trials, observing that particles <500 µm carried 1.7 × 10^4 CFU/cm^2 of *Salmonella*, compared to 0.9 × 10^4 CFU/cm^2 on larger particles. Mohammed (2025) examined microplastic interactions with pathogens in freshwater biofilm reactors and reported *E. coli* survival rates of 85% after 10 days on weathered PE compared to 40% in planktonic conditions. Cholewińska (2025) reviewed studies on microplastic biofilms and concluded that biofilms often contained *Listeria* and *Clostridium* species at relative abundances of 10–12%, significantly higher than surrounding water. Nayebi (2025) investigated physicochemical influences on biofilm formation using flow-cell experiments, reporting that nutrient enrichment increased pathogen counts by 2.5-fold and salinity adjustment led to 30% higher adhesion rates. Ahmed et al. (2025) analyzed microplastics from seafood processing wastewater and detected clinically relevant pathogens at concentrations ranging from 1 × 10^3 to 5 × 10^3 CFU per particle. Patel et al. (2025) evaluated pathogen transfer from microplastic biofilms to oysters using exposure assays, finding that *Vibrio* spp. levels increased from 2 × 10^3 to 1 × 10^4 CFU/g tissue over 7 days. Liu et al. (2025) used shotgun metagenomics on riverine microplastics and identified virulence and antibiotic resistance gene clusters, with ARG copy numbers ranging from 1.5 × 10^3 to 6 × 10^3 per particle. Cholewińska (2024) conducted a freshwater survey and found *Listeria* counts of 0.8–1.5 × 10^4 CFU/cm^2 on microplastic biofilms. McIlwraith et al. (2021) evaluated *E. coli* O157:H7 persistence under simulated sunlight, observing 70% survival on microplastic surfaces after 5 days, compared to <20% in water. Hoffman et al. (2022) examined microbial adhesion via atomic force microscopy, showing that surface roughness increased pathogen attachment by approximately 45%. Singh and Verma (2023) studied nutrient enrichment effects in microplastic biofilms, reporting that pathogen abundance increased from 1 × 10^3 to 4 × 10^3 CFU/cm^2 under high nutrient conditions. Oliveira and Santos (2023) conducted controlled exposure experiments in tilapia larvae and observed gut colonization with 1.2 × 10^4 CFU/g of pathogenic bacteria following ingestion of microplastic particles. Bayat et al. (2023) monitored microplastics in commercial shrimp and found 20–25% of ingested microplastics were colonized with

pathogenic bacteria, including *Vibrio* and *Listeria*, confirming a direct link to foodborne pathogen exposure.

Aim and Objectives of the Study

The aim of this study is to investigate microplastic biofilm formation in aquatic environments and assess their role in harboring and transmitting foodborne pathogens to aquatic food sources. The objectives were to:

i. characterize the microbial composition of biofilms formed on microplastics in aquatic environment;
ii. determine the survival of foodborne pathogens on microplastic biofilms under varying environmental conditions (temperature, salinity, and nutrient availability);
iii. evaluate the potential of microplastics to act as vectors for the transfer of foodborne pathogens to aquatic food sources (fish, shellfish, and other seafood);
iv. assess the influence of microplastic characteristics (size, shape, and polymer type) on biofilm formation and pathogen colonization.

II. MATERIALS AND METHODS

Sample Collection

Microplastic samples (3) were collected from selected aquatic environments (rivers, lakes, and coastal waters), using a plankton net (mesh size 300 µm) and manually from surface water. The collected microplastics were carefully transferred into sterile containers and transported to the laboratory on ice for immediate analysis.

Characterization of microbial composition of biofilms formed on microplastics

In the laboratory, visible debris and non-plastic materials were removed using sterile forceps. The microplastics were then rinsed gently with sterile phosphate-buffered saline (PBS) to remove loosely attached microorganisms while retaining the biofilm communities. Biofilms were detached from the microplastic surfaces by vortexing each sample in 10 mL of sterile phosphate-buffered saline (PBS) for 5 minutes, followed by sonication at 40 kHz for 3 minutes to ensure maximum recovery of attached microorganisms. The resulting suspension was serially diluted and plated onto selective and non-selective agar media (nutrient agar, MacConkey agar, and mannitol salt agar), to isolate and enumerate heterotrophic bacteria, coliforms, and Staphylococcus species. Plates were incubated at 30–37 °C for 24–48 hours, and colony-forming units (cfu) per particle were recorded. DNA was extracted from the biofilm suspensions using a commercial microbial DNA extraction kit according to the manufacturer's protocol. The 16S rRNA gene was amplified using universal primers (27F/1492R), and PCR products were sequenced. Sequences were analyzed using bioinformatics tools, QIIME2, to determine the bacterial composition and relative abundance within the biofilms. Phylogenetic analyses were performed to identify potential foodborne pathogens and their distribution across different microplastic types. Additionally, fluorescence microscopy using LIVE/DEAD staining was performed on a subset of biofilm samples to visualize biofilm structure and assess cell viability. Confocal laser scanning microscopy (CLSM) was used to examine biofilm thickness and spatial distribution on microplastic surfaces.

survival of foodborne pathogens on microplastic biofilms under varying environmental conditions

The biofilm-coated microplastics were placed into sterile microcosms containing artificial freshwater, adjusted to simulate different environmental conditions. Temperature conditions were set at 15 °C, 25 °C, and 35 °C; salinity was adjusted to 0.5‰, 15‰, and 30‰ using NaCl; and nutrient availability was varied by supplementing media with low (0.1% glucose), medium (0.5% glucose), and high (1% glucose) nutrient concentrations. Each treatment was conducted in triplicate and incubated for periods of 1, 3, 5, and 7 days.

Potential of microplastics to act as vectors for the transfer of foodborne pathogens to aquatic food sources

Aquatic organisms, tilapia (Oreochromis niloticus), shrimp (Litopenaeus vannamei), and mussels (Mytilus edulis), were obtained from aquaculture farms in Rumuolumeni and acclimatized in sterile

tanks containing artificial freshwater or seawater for 7 days prior to experimentation. Microplastic particles were introduced into experimental tanks at environmentally relevant concentrations (1–5 mg/L), and organisms were exposed for periods of 24, 48, 72, and 96 hours under controlled conditions (temperature: 25 °C; dissolved oxygen: 6–8 mg/L; light-dark cycle: 12:12 h). At each sampling interval, tissues of fish (gills and gastrointestinal tract), shellfish (digestive glands), and other seafood were aseptically dissected and homogenized in sterile PBS. Homogenates were serially diluted and plated on selective media: MacConkey agar for Escherichia coli, XLD agar for Salmonella spp., and Mannitol Salt Agar for Staphylococcus aureus. Plates were incubated at 37 °C for 24–48 hours, and colony-forming units (CFU/g tissue) were recorded to quantify pathogen transfer from microplastics to host tissues. Molecular confirmation was conducted by extracting DNA from tissue homogenates using a commercial DNA extraction kit, followed by quantitative PCR (qPCR) with species-specific primers to detect and quantify the transferred pathogens. Fluorescence microscopy with LIVE/DEAD staining was used to visualize pathogen attachment and viability within host tissues, and confocal laser scanning microscopy (CLSM) was employed to examine microplastic adherence and biofilm transfer onto gill and digestive surfaces.

Influence of microplastic characteristics (size, shape, and polymer type) on biofilm formation and pathogen colonization

Microplastic particles of varying characteristics were collected from aquatic environments. Particles were categorized by size (small: <500 µm; medium: 500–1000 µm; large: >1000 µm) and shape (fragments, fibers, and beads). All microplastics were sterilized by washing in 70% ethanol and rinsing with sterile phosphate-buffered saline (PBS) prior to experiments. Sterilized microplastics were incubated in aquatic microcosms containing natural water samples spiked with foodborne pathogens (Escherichia coli, Salmonella spp., and Staphylococcus aureus,) to allow biofilm formation over 7 days. The microcosms were maintained under controlled temperature (25 °C), light conditions (12:12 h light-dark cycle), and gentle aeration to mimic natural aquatic environments. After incubation, biofilms were detached from microplastic surfaces using vortexing and mild sonication in sterile PBS. The resulting suspensions were serially diluted and plated on selective and differential media to enumerate pathogen populations (CFU/particle) and assess microbial community composition. Colony counts were compared across microplastic types, sizes, and shapes to evaluate their influence on pathogen colonization.

III. RESULTS

Table 4.1: Microbial Composition and Biofilm Characteristics of Microplastics from Aquatic Environments

Sample ID	Type	Env	HB (cfu)	C. (cfu)	S. spp. (cfu)	Dominant Bacterial	Potential Pathogens	B. T (µm, CLSM)	C.V (%) (L/D)
MP-L2	PE Film	Lake	1.0×10^4	3.0×10^2	1.5×10^2	Proteobacteria (55%), Bacteroidetes (25%), Firmicutes (10%)	*E. coli*	14 ± 2	87
MP-C1	PS Fragment	Coastal	2.0×10^4	5.0×10^2	2.5×10^2	Proteobacteria (60%), Bacteroidetes (20%), Firmicutes (10%)	*Vibrio spp.*, *S. aureus*	20 ± 4	92
MP-C2	PP Pellet	Coastal	1.8×10^4	4.5×10^2	2.0×10^2	Proteobacteria (50%), Bacteroidetes	*Vibrio spp.*, *Salmonella spp.*	17 ± 3	89

						(30%), Actinobacteria (10%)			
MP-R1	PE Fragment	River	1.2×10^4	3.5×10^2	2.1×10^2	Proteobacteria (45%), Bacteroidetes (30%), Firmicutes (15%)	*E. coli*, *S. aureus*	15 ± 2	88
MP-R2	PP Pellet	River	9.5×10^3	2.8×10^2	1.8×10^2	Proteobacteria (50%), Actinobacteria (20%), Bacteroidetes (20%)	*Salmonella spp.*, *S. aureus*	12 ± 1.5	85
MP-L1	PET Fragment	Lake	1.5×10^4	4.2×10^2	1.2×10^2	Proteobacteria (40%), Bacteroidetes (35%), Firmicutes (10%)	*E. coli*, *Listeria monocytogenes*	18 ± 3	90

The results in Table 4.1 indicate that microplastics from different aquatic environments harbor diverse microbial communities and serve as substrates for biofilm formation. Polyethylene (PE), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET) particles all supported biofilms dominated by Proteobacteria, Bacteroidetes, and Firmicutes, with bacterial abundances ranging from ~9.5×10^3 to 2.0×10^4 CFU. Importantly, several microplastics were colonized by potential pathogens, including *E. coli*, *Salmonella spp.*, *Vibrio spp.*, *S. aureus*, and *Listeria monocytogenes*, suggesting that these particles can act as vectors for disease transmission in aquatic systems. Biofilm thickness (12–20 µm) and high coverage values (85–92%) observed via CLSM demonstrate that microplastics provide stable surfaces for microbial adherence and growth.

Table 4.2: Survival of Foodborne Pathogens on Microplastic Biofilms under Varying Environmental Conditions

Pathogen	Temp (°C)	Salinity (‰)	Nutrient Level	Day 1 (log CFU/)	Day 3	Day 5	Day 7	Observation
E. coli	15	0.5	Low (0.1%)	4.2	3.9	3.5	3.0	Gradual decline; low temp slows metabolism
E. coli	25	0.5	Medium (0.5%)	4.5	4.4	4.2	4.0	Moderate survival; optimal growth temp
E. coli	35	0.5	High (1%)	4.8	4.7	4.5	4.3	High temp + nutrients supports survival
Salmonella spp.	15	15	Medium (0.5%)	3.8	3.5	3.2	2.8	Survival reduced under moderate salinity
Salmonella spp.	25	15	High (1%)	4.5	4.4	4.2	4.0	Optimal survival at moderate temp & nutrients
Salmonella spp.	35	30	Low (0.1%)	4.0	3.6	3.2	2.7	High salinity + low nutrients reduces viability

Listeria monocytogenes	15	0.5	Low (0.1%)	4.3	4.1	3.9	3.6	Cold-tolerant pathogen shows slower decline
Listeria monocytogenes	25	15	Medium (0.5%)	4.6	4.5	4.4	4.3	Moderate salinity supports survival
Listeria monocytogenes	35	30	High (1%)	4.7	4.5	4.3	4.2	High temp + nutrients sustains viability
Staphylococcus aureus	25	0.5	Medium (0.5%)	4.5	4.4	4.2	4.1	Strong biofilm formation ensures survival
Staphylococcus aureus	35	30	High (1%)	4.8	4.7	4.6	4.5	High nutrients + temp favors persistence
Staphylococcus aureus	15	15	Low (0.1%)	4.0	3.7	3.5	3.2	Low nutrients + cold slows metabolism

The result in table 4.2 indicated that temperature, salinity, and nutrient availability strongly influence the survival of aquatic pathogens over time. *E. coli* and *Listeria monocytogenes* showed better persistence at moderate to high temperatures and nutrient levels, whereas low temperatures slowed their metabolism and caused gradual declines. *Salmonella spp.* survival was negatively affected by high salinity combined with low nutrients, highlighting its sensitivity to osmotic stress. *Staphylococcus aureus* demonstrated strong resilience under conditions favouring biofilm formation, with high temperature and nutrient levels promoting sustained viability.

Table 4.3: Potential of microplastics to act as vectors for the transfer of foodborne pathogens to aquatic food sources

Organism	Tissue	Exposure Time (h)	*E. coli* (CFU/g)	*Salmonella spp.* (CFU/g)	*S. aureus* (CFU/g)	qPCR Detection (Gene copies/g)	Observations (Fluorescence & CLSM)
Tilapia (*Oreochromis niloticus*)	Gills	24	3.2×10^2	1.8×10^2	2.5×10^2	5.0×10^3	Microplastics adhered to gill filaments; biofilm visible; mostly viable cells
Tilapia	GIT	24	4.5×10^2	2.2×10^2	3.0×10^2	6.8×10^3	Pathogens embedded in mucus layer; viable cells detected
Tilapia	Gills	48	4.0×10^2	2.0×10^2	3.2×10^2	7.2×10^3	Increased attachment; thicker biofilm on gills
Tilapia	GIT	48	5.2×10^2	2.5×10^2	3.5×10^2	8.5×10^3	Pathogen accumulation higher; viable cell clusters visible
Tilapia	Gills	72	4.5×10^2	2.2×10^2	3.8×10^2	7.8×10^3	Biofilm partially dispersed; pathogens still

							viable
Tilapia	GIT	72	5.5×10^2	2.8×10^2	4.0×10^2	9.0×10^3	Dense biofilm remnants; strong qPCR signals
Tilapia	Gills	96	4.0×10^2	2.0×10^2	3.5×10^2	7.5×10^3	Biofilm thinning; live/dead staining shows ~80% viable cells
Tilapia	GIT	96	5.0×10^2	2.5×10^2	4.2×10^2	8.7×10^3	Pathogens mostly viable; adhesion to intestinal mucus evident
Shrimp (*Litopenaeus vannamei*)	Digestive gland	24	2.5×10^2	1.5×10^2	1.8×10^2	4.2×10^3	Microplastics present in digestive tract; biofilm intact
Shrimp	Digestive gland	48	3.0×10^2	1.8×10^2	2.0×10^2	5.0×10^3	Pathogens increasing; live cells dominate
Shrimp	Digestive gland	72	3.5×10^2	2.0×10^2	2.2×10^2	5.5×10^3	Dense biofilm patches visible; viable clusters
Shrimp	Digestive gland	96	3.2×10^2	1.9×10^2	2.0×10^2	5.3×10^3	Biofilm partially detached; ~85% live cells
Mussels	Digestive gland	48	3.2×10^2	2.0×10^2	2.3×10^2	5.0×10^3	Biofilm stable; increasing pathogen load
Mussels	Digestive gland	72	3.5×10^2	2.2×10^2	2.5×10^2	5.5×10^3	Dense microplastic-biofilm aggregates observed
Mussels	Digestive gland	96	3.2×10^2	2.0×10^2	2.3×10^2	5.3×10^3	Biofilm partially disrupted; viable pathogens persist

The result in table 4.3 indicated that microplastics can serve as vectors for pathogen colonization in aquatic organisms, with colonization dynamics varying by organism, tissue type, and exposure time. In Tilapia, the gastrointestinal tract consistently harbored higher counts of *E. coli*, *Salmonella spp.*, and *S. aureus* than the gills, reflecting the mucus-rich environment that favors microbial attachment and biofilm formation. Biofilms on gills were initially thick but partially dispersed over time, yet a high proportion of viable cells persisted (~80%). Shrimp digestive glands exhibited gradual increases in pathogen load over time, with dense biofilm patches forming by 72 h, though partial biofilm detachment was observed at 96 h. Similarly, mussels maintained stable biofilms with increasing pathogen load initially, followed by partial biofilm disruption, while pathogens remained viable. qPCR detection confirmed that microbial DNA remained abundant throughout the exposure period, indicating persistent colonization even as biofilm structure changed.

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Table 4.4: Influence of Microplastic Characteristics on Pathogen Colonization (CFU/particle)

Microplastic Characteristic	Size	Shape	E. coli (CFU/particle)	Salmonella spp. (CFU/particle)	Staphylococcus aureus (CFU/particle)	Trend in Biofilm Formation
Polyethylene (PE)	Small (<500 µm)	Fragment	1.2×10^4	9.5×10^3	7.0×10^3	Moderate biofilm, surface area limits colonization
Polyethylene (PE)	Medium (500–1000 µm)	Fragment	2.0×10^4	1.5×10^4	1.2×10^4	Higher biofilm due to larger surface area
Polyethylene (PE)	Large (>1000 µm)	Fragment	2.5×10^4	2.0×10^4	1.5×10^4	Max biofilm formation, stable pathogen attachment
Polypropylene (PP)	Small (<500 µm)	Fiber	1.5×10^4	1.0×10^4	8.0×10^3	Fibrous shape promotes initial biofilm adherence
Polypropylene (PP)	Medium (500–1000 µm)	Fiber	2.3×10^4	1.8×10^4	1.3×10^4	Fibers support thicker biofilms
Polypropylene (PP)	Large (>1000 µm)	Fiber	3.0×10^4	2.5×10^4	1.8×10^4	Highest colonization due to surface complexity
Polystyrene (PS)	Small (<500 µm)	Bead	0.8×10^4	6.0×10^3	5.0×10^3	Smooth surface, less biofilm formation
Polystyrene (PS)	Medium (500–1000 µm)	Bead	1.5×10^4	1.1×10^4	8.5×10^3	Moderate biofilm formation
Polystyrene (PS)	Large (>1000 µm)	Bead	2.0×10^4	1.5×10^4	1.1×10^4	Larger beads allow more pathogen attachment

The results in table 4.4 demonstrated that microplastic characteristics such as size, shape, and polymer type strongly influence pathogen colonization and biofilm development. Larger microplastics generally supported higher pathogen loads due to increased surface area, with *E. coli*, *Salmonella spp.*, and *Staphylococcus aureus* all showing elevated CFU/particle on medium and large fragments or fibers compared to small particles.

Fibrous polypropylene (PP) exhibited the highest colonization, as the complex surface topography promotes initial microbial attachment and thicker biofilm formation. In contrast, smooth polystyrene (PS) beads showed comparatively lower pathogen loads, reflecting reduced surface roughness and limited biofilm development. Polyethylene (PE) fragments supported moderate to high colonization, with biofilm formation increasing with particle size.

IV. DISCUSSION

The results in Table 4.1 demonstrate that microplastics from diverse aquatic environments support rich microbial communities and serve as stable substrates for biofilm formation. Total heterotrophic bacterial counts ranged from 9.5×10^3 to 2.0×10^4 CFU, reflecting significant microbial colonization across polyethylene (PE), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET) particles. Biofilms were predominantly composed of Proteobacteria (40–60%), Bacteroidetes (20–35%), and Firmicutes (10–15%), indicating that these phyla preferentially adhere to synthetic surfaces. Several microplastic samples were colonized by potential pathogens, including E. coli, Salmonella spp., Vibrio spp., S. aureus, and Listeria monocytogenes, establishing that microplastics function as reservoirs and vectors for pathogenic bacteria. Biofilm thickness (12–20 µm) and high coverage values (85–92%) observed via CLSM further highlight that microplastic surfaces provide favorable microhabitats for bacterial attachment and growth. These observations are consistent with Amaral-Zettler et al. (2020), who reported that marine microplastics are colonized by diverse microbial communities, including pathogenic species, which persist and disperse through aquatic ecosystems. Similarly, Oberbeckmann et al. (2018) demonstrated that polymer type and surface characteristics significantly influence biofilm development, with rougher surfaces supporting thicker microbial layers. The presence of pathogens on PE, PP, PS, and PET in this study aligns with Kesy et al. (2019), who documented adhesion of E. coli and Salmonella spp. to microplastic debris in freshwater environments. Moreover, the dense and stable biofilms observed here corroborate the findings of Zettler et al. (2013), which reported that microbial aggregates on microplastic surfaces protect bacteria and facilitate survival in varying environmental conditions.

The results in Table 4.2 demonstrate that environmental factors, including temperature, salinity, and nutrient availability, significantly influence the survival of foodborne pathogens on microplastic biofilms. E. coli and Listeria monocytogenes exhibited enhanced persistence at moderate to high temperatures (25–35 °C) and nutrient-rich conditions, whereas low temperatures (15 °C) slowed metabolic activity, resulting in gradual declines in viable counts over the 7-day period. Salmonella spp. survival was markedly reduced under high salinity (30 ‰) combined with low nutrient levels (0.1%), reflecting the pathogen's sensitivity to osmotic and nutritional stress. In contrast, Staphylococcus aureus demonstrated high resilience under conditions conducive to biofilm formation, with elevated temperatures and nutrient concentrations sustaining pathogen viability over time. These observations align with previous studies emphasizing the role of environmental conditions in microbial persistence. Kesy et al. (2019) reported that E. coli on microplastic surfaces maintains viability longer at moderate temperatures and nutrient availability, consistent with the trends observed in this study. Oberbeckmann et al. (2018) demonstrated that temperature and nutrient enrichment enhance biofilm thickness and pathogen retention on polymer surfaces. Similarly, Amaral-Zettler et al. (2020) noted that biofilm-associated pathogens persist under varied aquatic conditions due to the protective matrix. Zettler et al. (2013) highlighted that microplastic biofilms provide physical shelter from environmental stressors, which preserves bacterial viability. Additionally, Wright et al. (2020) found that the combination of nutrient availability and ambient temperature determines the survival and dispersal of pathogens in aquatic systems. However, Reichert et al. (2021) found that Listeria monocytogenes survival decreases sharply under low temperatures despite biofilm formation, suggesting that matrix protection does not fully offset cold-induced metabolic slowdown. Chen et al. (2019) observed that high salinity significantly reduces Salmonella spp. viability on synthetic surfaces, in agreement with the decline seen in this study, but they reported more rapid die-off under fluctuating nutrient regimes. Harrison et al. (2014) argued that while biofilm formation enhances pathogen persistence, environmental stressors such as UV exposure or sudden temperature shifts can compromise survival.

The results in Table 4.3 demonstrate that microplastics act as vectors for the colonization and transfer of foodborne pathogens in aquatic organisms, with colonization dynamics influenced by organism

type, tissue, and exposure duration. In Tilapia (Oreochromis niloticus), the gastrointestinal tract consistently harbored higher counts of E. coli, Salmonella spp., and S. aureus compared to the gills, reflecting the mucus-rich environment that promotes microbial attachment and biofilm development. Biofilms on gills were initially dense but partially dispersed over time; nevertheless, a substantial proportion of viable cells persisted (~80%), indicating strong pathogen resilience. In Litopenaeus vannamei (shrimp), digestive glands exhibited gradual increases in pathogen load over 96 h, with dense biofilm patches forming by 72 h, although partial detachment occurred by 96 h. Similarly, mussels maintained stable biofilms with initially increasing pathogen load, followed by partial disruption, while pathogens remained viable. qPCR detection confirmed sustained microbial DNA abundance throughout the exposure period, demonstrating persistent colonization even as biofilm architecture changed. These observations are in line with previous studies indicating that microplastics provide suitable substrates for pathogen persistence and transfer in aquatic food webs. The high pathogen counts in tilapia gastrointestinal tissues tallies with the findings of Kesy et al. (2019), who reported that microplastics in freshwater facilitate the adhesion and survival of E. coli and Salmonella spp.. The persistence of biofilm-associated pathogens is consistent with the work of Oberbeckmann et al. (2018), which demonstrated that biofilm formation on synthetic polymers enhances microbial retention under environmental conditions. Similarly, Amaral-Zettler et al. (2020) highlighted that biofilms on microplastics create protective niches, preserving bacterial viability, which aligns with the sustained pathogen detection in shrimp and mussels in this study. Wright et al. (2020) emphasized the role of microplastic-mediated transport in increasing exposure of aquatic organisms to pathogens, which corresponds with the higher pathogen loads observed in mucus-rich tissues. Conversely, the partial detachment of biofilms in shrimp and mussels tallies with the observations of Reichert et al. (2021), who noted that biofilm structure can be disrupted over time due to physical flow or environmental stressors.

The results in Table 4.4 demonstrate that microplastic characteristics, including size, shape, and polymer type, significantly influence pathogen colonization and biofilm development. Larger microplastic particles generally supported higher pathogen loads, with E. coli, Salmonella spp., and Staphylococcus aureus all exhibiting elevated CFU/particle on medium and large fragments or fibers compared to small particles. This trend is consistent with the concept that increased surface area facilitates microbial attachment and biofilm maturation. Fibrous polypropylene (PP) particles exhibited the highest colonization levels, likely due to the complex surface topography of fibers, which promotes initial microbial adhesion and allows the development of thicker biofilms. In contrast, smooth polystyrene (PS) beads consistently supported lower pathogen loads, reflecting limited surface roughness and reduced biofilm formation. Polyethylene (PE) fragments demonstrated moderate to high colonization, with biofilm development positively correlated with particle size. These observations are consistent with prior studies indicating that microplastic surface properties are key determinants of microbial adherence and biofilm dynamics. Kesy et al. (2019) reported that bacterial colonization is enhanced on polymers with larger surface areas, supporting the observed increase in CFU/particle with medium and large PE and PP particles. Oberbeckmann et al. (2018) similarly noted that particle morphology, particularly fibrous structures, promotes biofilm thickness and pathogen retention, aligning with the high colonization seen on PP fibers in this study. Amaral-Zettler et al. (2020) emphasized that biofilm matrices on microplastics provide a protective microenvironment, enabling sustained survival of attached pathogens under varied conditions, which may explain the persistence of E. coli, Salmonella spp., and Staphylococcus aureus across different microplastic types. Zettler et al. (2013) highlighted that biofilm formation on microplastics shields microorganisms from physical and chemical stressors, supporting the observed trend that surface complexity enhances microbial colonization. Wright et al. (2020) further suggested that particle size and morphology directly influence biofilm establishment, dispersal potential, and pathogen viability, consistent with the higher CFU/particle recorded on larger fragments and fibers in the present study. However, differences in polymer chemistry also play a role in microbial adherence and biofilm formation. The

relatively low colonization observed on smooth PS beads aligns with Harrison et al. (2014), who argued that surface roughness and hydrophobicity modulate initial microbial attachment, while smooth surfaces limit biofilm expansion. Similarly, Chen et al. (2019) reported that polymer-specific properties, including hydrophobicity and surface charge, affect Salmonella spp. colonization, which agrees with the lower CFU/particle recorded on PS in comparison to PE and PP. These findings suggest that both physical (size, shape) and chemical (polymer type) characteristics of microplastics interact to determine pathogen load and biofilm formation potential.

V. CONCLUSION

The study demonstrates that microplastics in aquatic environments harbor diverse microbial communities and serve as substrates for pathogen colonization. Biofilm formation was influenced by particle size, shape, and polymer type, with larger and fibrous microplastics supporting higher pathogen loads. Potential pathogens, including E. coli, Salmonella spp., S. aureus, and Listeria monocytogenes, were detected on microplastics and transferred to aquatic organisms such as Tilapia, shrimp, and mussels. Environmental conditions, including temperature, salinity, and nutrient levels, further affected pathogen survival, with higher temperatures and nutrient availability favoring persistence.

VI. RECOMMENDATIONS

Based on the result of the finding, the following were recommended;

i. Environmental protection agencies, such as the National Environmental Standards and Regulations Enforcement Agency (NESREA) and local water management authorities, should regularly monitor aquatic environments for microplastic contamination to identify high-risk areas for pathogen accumulation.
ii. Industrial regulators, municipal waste management authorities, and environmental NGOs should implement policies and interventions to minimize microplastic release from industrial, agricultural, and domestic sources, including stricter waste management practices and public awareness campaigns.
iii. Aquaculture farms, seafood processors, and food safety authorities should adopt good handling and processing practices to reduce the risk of pathogen transfer from microplastic-contaminated water to fish, shrimp, and shellfish.
iv. Academic institutions, research centers, and funding agencies should prioritize studies on the long-term impacts of microplastic-associated pathogens on human health and aquatic food safety, including mechanisms of biofilm-mediated pathogen transfer

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