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Exposure to High-molecular-weight Polyvinyl Chloride Alters Bacterial Diversity in the Gut Microbiota of the Wistar Rat

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Abstract: The physical and chemical characteristics of microplastics make it easier for contaminants to adhere to the surface of the particles, acting as a vehicle for toxins to reach organisms after ingestion. The "most microbiome" comprises all the microorganisms present in our bodies as a whole because it has a big surface area and provides nutrient-rich components for the digestive system's germs. In this investigation, metagenome analysis was used to determine the impact of long-term administration of High-Molecular Weight-Polyvinyl Chloride microplastics to young Wistar rats on the gut microbiota. Forty adult rats in total were employed, with 15 first-group and 15 second-group experimental groups and 10 controls. Pellets made specifically for feeding rats are produced. Following the procedure, the rats were anaesthetised with ketamine and xylasine before being dissected. Due to the small number of samples, alpha diversity in the gut metagenome study did not demonstrate statistically significant variations, but it did illustrate differences in bacterial diversity and density. In particular, it has been discovered that bacterial diversity is higher in experimental groups. According to the control groups, in the assay groups, the intestinal microbiome, dominated by Escherichia coli, Shigella, and Lactobacillus, was assessed as an increase in metabolic pathways related to microplastic exposure and pathogenicity in general. The findings demonstrate the necessity for extreme caution in the manufacture and use of plastics that pose a risk to the welfare of living things.

Keywords: HMW-PVC, intestine, krona, metagenome, microbiota

1. Introduction

Our air, water, and land are being contaminated by the rapid growth in population, technological advancements, and the diverse goods generated as a result. The most prevalent and harmful type of this pollution is undoubtedly plastic. They are classified as microplastics (MPs) by synthetic hard polymeric matrices of primary or secondary origin insoluble in water. Their sizes range from 1 µm to 5 mm. The polyethylene particles, polyester fibers, textiles, plastic bags, and packaging that makeup MPs, the most significant means of use in today's world, enter the ocean. Marine organisms have been affected by microscopic plastic rashes (MPs), which represent serious hazards (Yurtsever 2019).

A dynamic mixture of polymers and additives in which organic materials and pollutants can be joined progressively to generate an eco-corona is a new and more thorough definition of nano- (NP) and microplastic (MP) pollution in literature. Plastics cause the density and surface load of the particles in the structure to rise, and other compounds' bioavailability and toxicity can change (Galloway et al. 2017).

Numerous studies have looked into how model chemicals affect the microbiomes of individual species and artificial food chains in recent years (Carbery et al. 2018). The genome that humans and other communally dwelling species carry is known as a "microbiome", and it exhibits qualities that assist our body in accomplishing duties that cannot be done. The term "microbiota" refers to all the microorganisms found in our bodies. Therefore, the human body needs a healthy digestive system to maintain a healthy existence. Intestinal microbiota helps to do this (Karatay 2019).

The human microbiome project aimed to conduct a multidisciplinary investigation into the distribution, evolution, and consequences of the bacteria that make up the human microbiota. It was initiated in 2008 in the United States and involved European and Asian nations. The majority of the human microbial population is made up of the microorganisms that live in the gut, which is home to at least 7000 different species (Karatay 2019), 1000 different bacterial species, and almost 150 times as many genes as the human genome (Qin et al. 2010).

Because of its enormous surface area and plenty of nutrients for microbes, the digestive tract, particularly the colon, is where most of the microbiota is found. More than 70% of the microorganisms in our bodies are found in the colon alone (Ley et al. 2005, Qin et al. 2010), which also offers a favourable environment for colonisation in the epidermis, genitourinary system, and respiratory system (Coleman et al. 2018).

A particular mix of helpful and dangerous microorganisms can be found in the microbiome (Whitman et al. 1998). The microbiome's inhabitants often form a symbiotic field and are crucial to many bodily processes. These include controlling cellular growth, restoring tissues after injury, maintaining barrier functions in systems, producing vitamins, short-chain free fatty acids (SCFA), conjugate linoleic acids (CLA), amino acid synthesis, bio-transforming bile acids, fermenting non-digestible nutrients, hydrolysis, ammonia syntheses, detoxification, and inducing, developing, and modulating immune responses to foreign substances and microbes (Neish 2014).

The pathogenesis and treatment of several disorders have been linked to bacteria in the human gastrointestinal system in recent years (Küçük & Ülger 2019). Microbial dysbiosis, defined as the decline of beneficial bacteria and the development of harmful bacteria, has been linked to metabolic illnesses like obesity, diabetes, cardiovascular disease, celiac disease, colon-gastric cancer, and celiac disease. According to studies, the microbial metabolite imidazole propionate may play a role in the aetiology of type 2 diabetes (Qin et al. 2010, Koh et al. 2018).

Additionally, microbiota dysbiosis demonstrated that some inflammatory immune disorders, such as inflammatory bowel disease (Morgan et al. 2012), Crohn's disease, rheumatoid arthritis (Scher et al. 2013), multiple sclerosis (Jangi et al. 2016), and neurodegenerative illnesses like Alzheimer's disease (Vogt et al. 2017) and Parkinson's disease (Fasano et al. 2015), and amyotrophic lateral sclerose (AML) (Blacher et al. 2019), in ocular conditions as autoimmune, age-related macular degeneration (AMD), glaucoma (Gong et al. 2020), and diabetic retinopathy (DR) (Bai et al. 2022). Additionally, it was discovered that the gut microbiota was linked to the development of tumors and melanoma (Riquelme et al. 2019).

The microbiota is a virtual organ that is expressed differently based on endogenous and exogenous factors such as geographic origin, genetics, mode of birth, age, way of life, diet, use of antibiotics, and prior illnesses (Karatay 2019).

The formation of the gut microbiota in humans starts at birth (Dominguez-Bello et al. 2010). Baby food (Escherichia coli, Clostridium difficile, Bacteroides fragilis, and Lactobacillus) and breast milk (Bifidobacteria) cause a range of microflora. 90% of the flora in healthy individuals is made up of the gram-positive Firmicutes (Clostridium, Eubacterium, Ruminococcus, Butyrivibrio, Roseburia, Anaerostipes, and Faecalibacteriom), gram-negative Bacteroidetes, Proteobacteria, and gram-positive Actinobacteria (Bifidobacterium genus). 60% of all flora are Firmicutes, 10% are Bacteroidetes, and 10% are Actinobacteria. Viruses, fungi, and several bacteria-like eukaryotic microorganisms comprise most of the human microbiome (Karatay 2019).

Metagenome analysis has not yet been used to investigate the changes in the microbiota that MPs can cause, especially in long-term treatment. Our study used metagenomic sequencing to ascertain the microbial makeup of young rats following prolonged exposure to HMW-PVC.

2. Material and Method

The Local Ethics Committee of the Cukurova University Medical Sciences Experimental Search and Application Center approved the experimental protocols (Decision No: 3 on March 18, 2021). The NIH Guide for Care and Use of Animals was followed when conducting the study.

2.1. Test Substances

Sigma-Aldrich provided the microplastic particles of High-Molecular-Weight Polyvinyl Chloride (HMW-PVC) in powder form, catalogue number 81387; 2 (Jovanović et al. 2018).

The Cukurova University Experimental Application and Research Center obtained the feed from a facility that produces pellets specifically for this purpose. Two different doses of HMW-PVC were evenly distributed to create pellet feeds.

2.2. Animals

In this study, 40 adult rats of the genus Wistar (their weight was 200±20 g) were used and were bred in the Cukurova University Center for Experimental Application and Research. Food and water were given ad libitum. The room temperature was fixed at 21±2°C. Animals were housed in a room with a 12-hour light/dark cycle. A 12-hour light/dark cycle chamber was employed to hold the animals.

2.3. Assay Procedure

In the 8-week study, there were 3 groups: 10 wistar rats served as the control group and were fed normal pellets; 15 wistar rats served as experimental Group 1 were fed pellets containing 1% of their weight in HMW-PVC; and 15 wistar rats served as experimental Group 2 were fed pellets containing 2% of their weight in HMW-PVC. All groups received unlimited food. The rats were dissected via dissection under ketamine-xylazine anaesthesia at the end of eight weeks, and metagenome analysis was carried out in the intestine.

2.4. DNA extraction from gastrointestinal fluid Samples

DNA was extracted using QuickGene (Kurabo, Japan) Tissue DNA Extraction Kit. First, a 250-l MDT solution-filled homogenisation tube was filled with a 25-mg intestine sample. The samples were homogenised by adding 15 mg of 0.1-mm zirconium beads. The tubes were rotated twice for 120 seconds at 5000 rpm. 25 l of EDT (Proteinase K) solution was added to the tube after homogenisation, and it was then incubated at 56°C for 60 minutes. The samples were then centrifuged at room temperature for 10 minutes at a speed of 15,000 g. 180 l of LDT solution was added to 200 l of supernatant after it had been transferred to a sterile 1.5 ml microcentrifuge tube, vortexed for 15 seconds, and then incubated at 70°C for 10 minutes. The tubes were vortexed for 15 seconds after being filled with 240 l of cold ethanol. After being promptly transferred to QuickGene (Kurabo) columns, the entire volume inside the microcentrifuge tube underwent three washes with 750 l of WDT solution. The process was completed by soaking QuickGene (Kurabo) columns in 200 l of CDT solution and extracting 50-60 ng of genomic DNA into a brand-new, sterile 1.5 ml microcentrifuge tube.

2.5. Illumina sequencing of 16S rRNA genes

Extracted DNA was amplified using the 16S V3-V4 region primers (341F: 5'-CCTAYGGGRBGCASCAG and 860R: 5'-GGACTACNNGGGTATCTAAT). Indexes were used to create the libraries. Pooled libraries were cleaned using a specific size choice following the manufacturer's approach (AMPure XP, Beckman Coulter). Sequencing was performed after library preparation, utilising Illumina MiSeq technology.

2.6. Metagenomic bioinformatics analysis

Pair-end Illumina reads (2x250; target region is around 450bp) were imported into the qiime2 environment (Bolyen et al. 2019). Every sample had a sequencing depth of over 100X (for 1 sample, an average 100,000 read), and no samples were removed from the analysis. Quality clipping, chimera identification, and read cleaning were implemented using the Qiime2 (Callahan et al. 2016). Low-quality reads with Phred score <30. Amplicon Sequence Variants (ASV) were mapped to the Silva 138 database following Dada2 (Schloss 2021).

The Phyloseq (McMurdie & Holmes 2013) object was created in the R 4.1 environment utilising the qiime2 artifact file. The results of the alpha diversity assessment, which examined the variety of related taxonomic units in a sample, were interpreted using three different indices: Chao1. The P values between groups were calculated using the Kruskal-Wallis test. To pinpoint particular changes across groups, differential abundance analysis (Deseq2 R pack) was employed (Love et al. 2014). A linear discriminant analysis effect size (LEFSe) investigation was conducted between groups to show statistically significant abundance differences between taxonomies(Segata et al. 2011).

3. Results

40 Wistar rats were used in this study. In an unpublished study we conducted in parallel with this study (Control (10) + Group 1 (15) + Group 2 (15), weight differences were observed in the control and some treatment groups.

The study generated data from 8 samples, including 2 controls and 3 from each of Group 1 and Group 2. All sequencing information is given in Table 1.

Table 1. The sequencing results of each sample

Sample ID	Forward sequence count	Reverse sequence count	bp read	Mbp read
Control				
1	70674	70674	35337000	35.34
2	51407	51407	27703500	25.70
Group 1				
1	123364	123364	61682000	61.68
2	105032	105032	52516000	52.52
3	15140	15140	7570000	7.57
Group 2				
1	73983	73983	36991500	36.99
2	72637	72637	36318500	36.32
3	12541	12541	6270500	6.27

3.1. Analysis of Microbial Alpha Diversity

Alpha diversity studies (Chao1, OTU) in control and experimental groups revealed changes in bacterial diversity and density in the experimental groups relative to the control, but these differences were not statistically significant. The following specific distinctions exist between the two groups.

Although species differences are similar, alpha diversity studies reveal the following variations in bacterial relative density between the control and experimental groups: The detected species, Chao1, Shannon, and Simpson indices, as well as any changes between the Control, Group 1, and Group 2 were not statistically different (Figure 1A, B, and C). The observed species in Group 2 seemed to be much lower than those in control and Group 1 (Figure 1D) ($p > 0.05$).

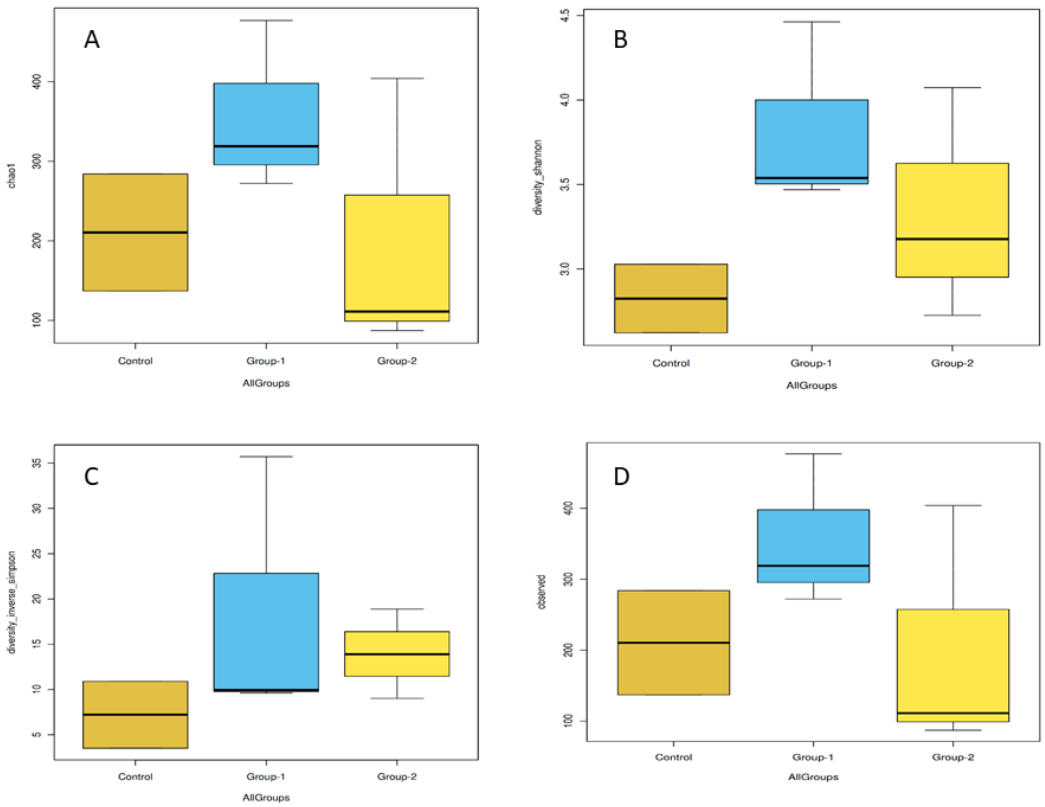

Fig. 1. Within-sample diversity measured by Chao1 index (A) Shannon diversity (B) Inverse-Simpson Index (C) Observed species (D) Kruskal-Wallis test was performed to analyse statistical significance

The main bacterial species found in the control group are: Turicibacter, p value: 5.42362145688901E-06; Lachnospiraceae, p rate: 1.52152086163749E-05; and Alloprevotella, p amount: 1.511520 861637 49E-05.

The main bacterial species found in the experimental Group 1 and 2 are: Blautia, p value: 2.18308660143668E-06; Ruminococcaceae, p rate: 2.18308660,1436668E-06; Eubacterium [hallii_group], p amount: 2.76082322972837E-06.

3.2. Composition of Microbial communities

In keeping with 16S RNA based on the findings (Figure 2) and krona charts (Figure 3), species histograms were created for the control and test groups at the levels of phylum, class, order, family, and genus.

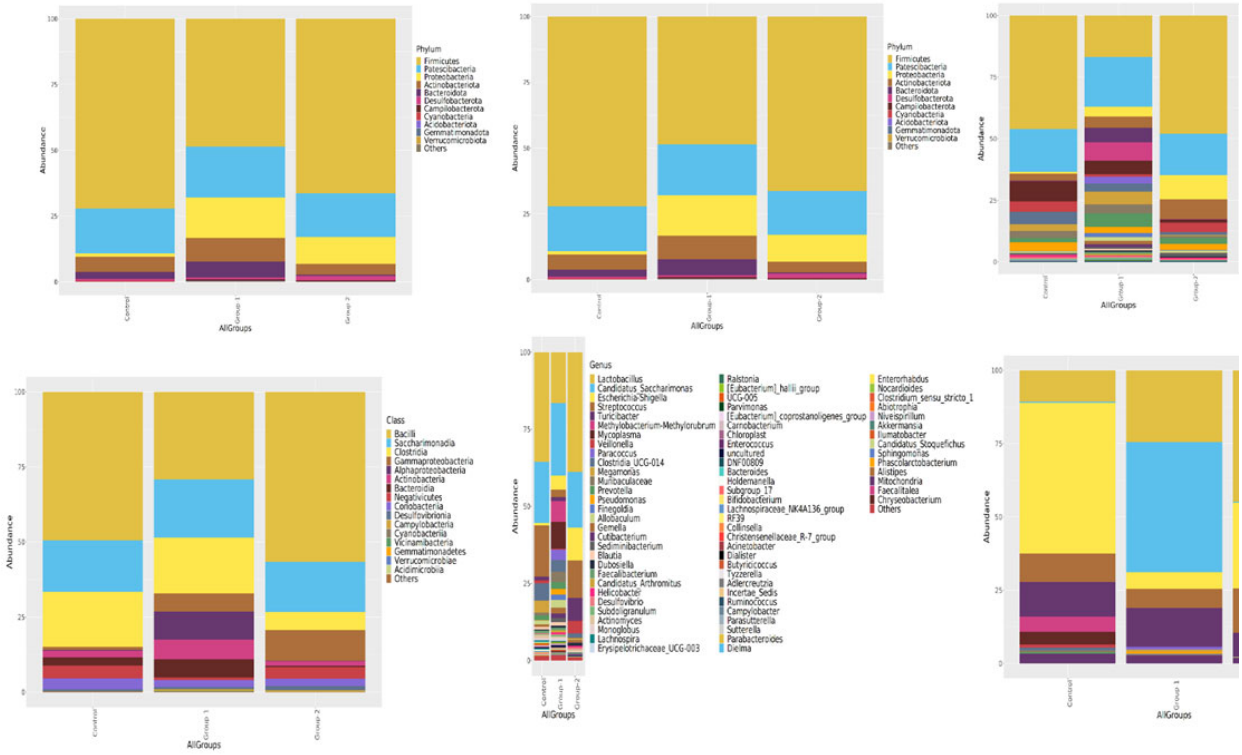

Fig. 2. Composition of microbial communities. Phylum level (A); Class level (B); Order level (C); Family level (D); Genus level (E); Species level (F)

Firmicutes (69.14% vs. 55%, 63%), Patescibacteria (20.27% vs. 27.17%, 15.49%), Actinobacteriota (6% vs. 5.38%, 4.73%), and Bacteroidota (2.43% vs. 3.77%, 0,53%) were the top four phyla of bacteria in terms of relative abundance in control groups and test Groups 1 and 2.

Bacilli (47.99% vs. 31,01% vs. 50,82%), Saccharimonadia (20.05% vs. 27.18% vs. 15.57%), and Clostridia (17.83% vs. 23.57% vs. 7,39%), respectively, were the top bacteria at the class level in control and assay Groups 1 and 2.

Lactobacillales (44.46%), Saccharimonadales (20.31% vs. 27.60%, 15.73%), Lachnospirales (7.18% vs. 7.10%, 1.33%), Clostridia (5.77% vs. 4.41%, 1,51%), and Veillonellales-Selenomonadales (3.37% vs. 1.14%, 5.17%) were the top bacteria at the order level in control and assay Group 1 and Group 2.

Lactobacillaceae (30.68% vs. 18.09%, 1.34%), Saccharimonadaceae (20.50% vs. 27.97%, 15.84%), Streptococcaceae (13.83% vs. 2.54%, 14.17%), and Clostridia (5.82% vs. 4.47%, 1.52) were the top bacteria at the family level in control and assay Groups 1 and 2.

Relative abundance at the genus level in control and assay Groups 1 and 2 were Lactobacillus (34.60% vs. 21.46%, 27.02%), Candidatus saccharimonas (23.10% vs. 33.17%, 0%), Streptococcus (15.56% vs. 2.99%, 15.3%), and Clostridium (6.57% vs. 5.3%, 1.6%); top bacteria at the species level in control and assay Group 1 and Group 2. As relative abundance t species level in control and assay Groups 1 and 2 were: Streptococcus hyointestinalis (52.13% vs. 7.3%, 30.75%), Lactobacillus faecis (13.30% vs. 19.06%, 5.79%) respectively. Further details are given in Table 2 and Figure 2.

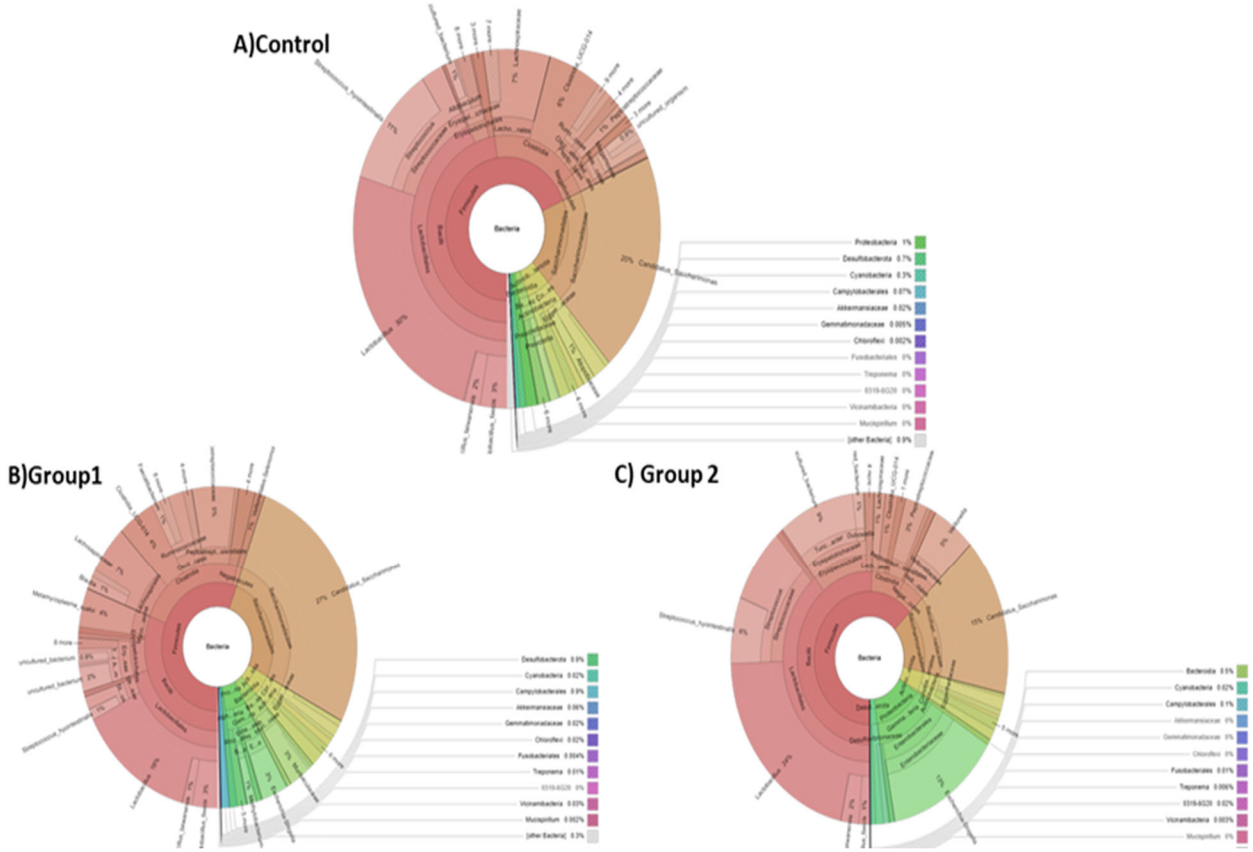

Fig. 3. Krona charts showing the phylum-level to species-level abundance, diversity and distribution of bacterial populations in Control, Group 1 and Group 2. Phylum level (A); Class level (B); Order level (C); Family level (D); Genus level (E); Species level (F)

Table 2. Relative abundance of the top bacteria at phylum level and percentage of control and assay group after exposure to HMW-PVC microplastics

Phylum	Control [%]		Group 1 [%]		Group 2 [%]	
1	Actinobacteriota	6.043	Acidobacteriota	0.03	Acidobacteriota	0.003
2	Bacteroidetes	2.437	Actinobacteriota	5.387	Actinobacteriota	4.73
3	Campilobacterota	0.069	Bacteroidetes	3.777	Bacteroidetes	0.539
4	Chloroflexi	0.002	Campilobacterota	0.885	Bdellovibrionota	0.02
5	Cyanobacteria	0.346	Chloroflexi	0.025	Campilobacterota	0.115
6	Desulfobacterota	0.705	Cyanobacteria	0.024	Cyanobacteria	0.024
7	Firmicutes	69.143	Deferribacterota	0.002	Desulfobacterota	2.063
8	Gemmatimonadota	0.005	Desulfobacterota	0.89	Firmicutes	63.013
9	Patescibacteria	20.027	Firmicutes	55.703	Fusobacteriota	0.012
10	Proteobacteria	1.207	Fusobacteriota	0.004	Patescibacteria	15.491
11	Verrucomicrobiota	0.016	Gemmatimonadota	0.019	Proteobacteria	13.984
12			Patescibacteria	27.171	Spirochaetota	0.006
13			Proteobacteria	6.007		
14			Spirochaetota	0.011		
15			Verrucomicrobiota	0.065		

4. Discussion

An ever-increasing volume of plastic is manufactured. All living creatures are at risk from environmental pollution since it can linger for decades in the air, soil, and water (Tibbetts 2015). It is estimated that each year, 8 million tons of plastic reach the ocean. In addition, 5.25 trillion plastic particles are thought to be present in the ocean's surface waters right now (Smith et al. 2018). Poor plastic waste management practices, high global demand for plastic goods, and continued manufacturing growth have all contributed to the accumulation of plastic debris in the world's oceans (Lehtinemi et al. 2021).

Numerous investigations conducted since 2010 have produced conflicting results about the risks and advantages of microplastics(Gündogdu 2018, Karami et al. 2018, Silva et al. 2018).

The gut microbiome is now recognised as having a considerable impact on both health and disease, as well as the pathophysiological mechanisms we are only now beginning to comprehend. Most contemporary microbiome observational research is limited to finding linkages and correlations rather than causations, even in large-scale longitudinal datasets (Ling et al. 2022).

We still don't fully understand the potential impacts of long-term intake and breathing of microplastics found in food. This study used metagomic analysis to assess the impact of chronic food intake of an untested kind of microplastic (HMW-PVC) on the intestinal microbiota in rats.

Since most of the gut microbiome components are anaerobic bacteria with incredibly robust cultures, the analytical approach primarily relies on "16S rRNA genes" (Waldor et al. 2015). Using 16S rRNA sequencing, typical Cytophaga-Flavobacterium Bacteroides (CFB) microorganisms have been discovered in the intestines of mice, rats, and humans (Mandal et al. 2015).

In a 2020 study, Zhou et al. identified the gut microbiota of postpartum depression (PPD) and Healthy Control (HC) patients using high-throughput sequencing of the 16S rRNA gene. The results showed that the richness and composition of the gut microbial communities in the PPD and HC groups were very different. They found that the HC group contained large populations of numerous butyrate-producing species, including Faecalibacterium, Phascolarctobacteria, and Butyricicoccus. In the PPD group, Escherichia coli and Enterococcaceae overgrowth were also discovered. Furthermore, there was no obvious distinction between the PPD and HC groups regarding variety (Zhou et al. 2020).

Other studies revealed that people with Major Depressive Disorder (MDD) have decreased microbial diversity (Dantzer et al. 2018). Hu et al. (2019) found significant differences between bipolar patients and healthy controls (HCs) in the variety of their gut flora.

The Lactobacillus genus, which is prevalent in the human intestine, is thought to have a key role in regulating the level of many metals, including selenium, in human cells, according to a study looking at the relationship between thyroid problems and the microbiome. Since these bacteria are required to create iodothyronine deiodinases, a decrease in bifidobacterium and lactobacillus levels can cause thyroid dysfunction (Ferreira et al. 2021).

A study on Graves disease (GD) patients discovered that both gaita samples and graves patients had a slight, but not statistically significant, increase in microbial diversity and wealth. Compared to healthy controllers, GDs were shown to have lower levels of Firmicutes and significantly greater levels of Bacteroidetes and Actinobacteria (Liu et al. 2022).

Studies using both human and animal models have demonstrated how obesity changes the flora in the gut. When the gut microbes of lean, wild-type, and obese mice were examined, the abundance of the phyla Bacteroidetes and Firmicutes differed. Ley et al. found that the Firmicutes: Bacteroidetes ratio, in particular, had a positive connection with obesity (Ley et al. 2005). Turnbaugh et al. (2006) also looked at the gut microbiota of lean and obese mice and found a link between diet-induced obesity and a rise in Firmicutes abundance. However, in animals with diet-induced obesity, the differences were connected to the growth of the Mollicutes class, a specific subclass of the Firmicutes phylum.

Based on alpha diversity analyses (Shannon, Simpson, and OTU) in our work, young rats exposed to HMW-PVC for 8 weeks displayed bacterial density and diversity variations. In particular, it was discovered that the experimental group's bacterial diversity was higher (Figure 1) than control.

The makeup of microbial communities in each group (sort and corresponding ratio) at various relative abundance levels were shown via histograms depicting species' relative abundance (Table 2, Figure 1).

According to sample-based differences, Group 1's gut microbiota diversity was noticeably higher than that of Groups 2 and the control group (Figure 2). This shows that Group 1 is more susceptible to bacterial transmission.

Numerous variables, such as diet, age, and others, might affect the bacterial microbiome. Numerous studies have linked a range of autoimmune and metabolic diseases to intestine dysbiosis (Le Chatelier et al. 2013).

The Krona analysis in our study paralleled the composition of the intestinal microbiome from the Filum to the genus levels and the overall organisation of the gut microbiota (Figure 3).

All our results demonstrate that chronic MP use promotes phylogenetic diversity in the mammal rat intestine. Due to the lack of comparable studies with HMW-PVC, we could not compare this study with them. However, the findings of other researchers who have discovered that Xenobiotics and microplastics can cause a range of diseases support the conclusions of this study.

Gram-positive Firmicutes (Clostridium, Eubacterium, Ruminococcus, Butyrivibrio, Roseburia, Anaerostipes, and Faecalibacterium), gram-negative Bacteroidetes, Proteobacteria, and gram-positive Actinobacteria (Bifidobacterium genera) make up 90% of the intestinal flora in healthy people under normal circumstances. According to researchers, Actinobacteria make up 10%, Bacteroidetes 10%, and Firmicutes 60% of the total flora (Küçük & Ülger 2019).

In our study, the major bacterial filaments that predominated in the rat control group were Furmicites (69%), Patescibacteria (20%), Actinobacteriota (6%), and at least Bacteroidetes (2.43%). In HMW-PVC exposed Group 1, 55, 27.17%, 5.38%, and 3.77% of the same filaments, respectively. In Group 2, it was detected 63%, 15.49%, 4.73%, and 0.53%, respectively.

Some bacteria known as furmicutes are found in the human intestines. Numerous species of the Firmicutes phylum create butyrate to maintain colon health. In our experimental groups, the Firmicutes phylum, which was the most prevalent in the control group, dropped.

The redundant metabolic, cellular, and stress-response activities have been significantly reduced in the Patescibacteria superphylum while keeping important capacities, such as digesting genetic information (Tian et al. 2020). The phylum Bacreiodetes increased in Group 1 while declining in Group 2, similar to the phylum Patescibacteria.

Using starch to break down complex polysaccharides, which encourages the growth of GALT and a fully formed immune system, is a benefit of the Bacteroides phylum. It prevents microorganisms from colonising the GI tract, encourages a healthy immune system, helps people avoid allergies and asthma, and may even be able to prevent obesity. According to Wexler et al. (2007), Bacteroides can cause bacteremia and infection after colonic contamination of the abdominal cavity and tissue (Wexler 2007). Group 1 also revealed an increase in the Patescibacterium phylum and the Bacreiodetes phylum, while Group 2 revealed a decline.

Actinobacteria, one of the four major phyla, are crucial for preserving gut homeostasis even though they only comprise a small percentage of the gut microbiota. Classes of bacteria of this phylum, especially Bifidobacteria, are frequently used as probiotics and have been proven successful in treating a range of clinical illnesses (Binda et al. 2018).

This phylum dropped in our study's experimental groups. This finding demonstrates that the intestinal homeostasis and intestinal microbiota of the rats in the experimental groups receiving HMW-PVC were altered.

Escherichia coli and Shigella, two pathogenic species, significantly increased in the gut microbiota of the HMW-PVC MP-exposed group compared to control (0.72%), Group 1 and 2, respectively (3.65% and 14.72%), whereas Firmicutes, a beneficial bacteria, significantly decreased (55.70 and 63.01%).

Escherichia coli and Shigella in the gut are associated with low short-chain fatty acid (SCFA) concentrations, an increase in metabolic pathways linked to pathogenicity, and the production of compounds associated with endotoxemia (Baltazar-Díaz et al. 2022).

The probiotic bacteria of the phylum Firmicutes and genus Lactobacillus are the most significant in the gut microbiome. The human body is affected by various inflammatory diseases that affect the intestines, lungs, heart, bones, or neurological tissues. Research continues to support the significance of Lactobacillus spp. and its components in influencing immunological responses (including metabolites, peptidoglycans, and/or surface proteins). To accomplish this, the gastrointestinal tract and other distant organs primarily exchange immunological signals (Rastogi & Singh 2022). Additionally, the rise in lactobacillus raised the possibility that these bacteria could work as a modulator to prevent intestinal damage caused by the immune system. Escherichia coli and Shigella pathogenic germs were shown to be on the rise in our study.

The study's inclusion of uncultivated species showed a metagenome gap. Therefore, in comparative study research, comparing the results of the metagenomic and culturomic analyses, we show that the use of culturomics allows the culture of organisms corresponding to sequences previously not assigned (Xie et al. 2021).

5. Conclusion

The gut microbiota of Wistar rats exposed to microplastic has a pathogenic or inflammatory environment. Considering utilising and recycling plastics is crucial since intestinal dysbiosis may develop from microplastic exposure. These results showed that diverse bacterial species and densities were present in the stomachs of rats exposed to long-term MP. Future microbiota studies involving all microplastics and nanoplastics will provide us with a wealth of insights, keeping with Hippocrates' adage from many years ago that "all diseases begin in the gut." Genome annotation studies further demonstrated that HMW-PVC MP exposure led to a significant

'functional dysbiosis' of the gut microbiota, impacting, among other things, the metabolic pathway, the regulatory pathway, and the production of secondary metabolites. Analysis of the human genome can also be used to predict the potential damage that xenobiotics may do to the mammalian body.

In the study, it was observed that bacterial diversity increased in the experimental groups. Additionally, the presence of organisms that could not be cultured in all groups seems to limit the metagenome analysis.

Statements and Declarations

Conflict of interest

There are no conflicts to declare.

Financial Interests

The authors declare they have no financial interests.

Author Contribution

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by [ABP], [ME], [BK], [SK], [TE] and [NE]. The first draft of the manuscript was written by [ABP, ET], and all authors commented on previous versions. All authors read and approved the final manuscript.

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