# Identifying macroplastic pathobiomes and antibiotic resistance in a subtropical fish farm

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#### Abstract :

Macroplastics are ubiquitous in aquaculture ecosystems. However, to date the potential role of plastics as a support for bacterial biofilm that can include potential human pathogenic bacteria (PHPB) and antibiotic-resistant bacteria (ARB) has been largely overlooked. In this study, we used a combination of metabarcoding and standard antibiotic susceptibility testing to study the pathobiome and resistome of macroplastics, fish guts and the environment in a marine aquaculture farm in Mauritius. Aquaculture macroplastics were found to be higher in PHPB, dominated by the Vibrionaceae family (0.34 % of the total community), compared with environmental samples. Moreover, isolates from aquaculture plastics showed higher significant multiple antibiotic resistance (MAR) compared to non-plastic samples of seawater, sediment and fish guts. These results suggest that plastics act as a reservoir and fomite of PHPB and ARB in aquaculture, potentially threatening the health of farmed fish and human consumers.

### Highlights

► The plastic bacteriome harbors more potential pathogens than environmental communities. ► Aquaculture plastic bacteria show a higher MAR index than non-plastic bacteria. ► Plastic can act as a reservoir for pathogenic and antibiotic-resistant bacteria. ► The fish and macroplastic bacteriome share certain pathogens. ► A nearby river can increase the risk of pathogen introduction in a fish farm.

Keywords : Plastisphere, Aquaculture, Pathogen, Antibiotic, resistance

#### 41 Introduction

Plastic pollution is a worldwide scourge that particularly affects marine environments, where 42 debris is ubiquitous (Chiba et al. 2018; Galgani et al. 2020). It is estimated that more than one 43 million tons of plastic waste enter oceans just from rivers each year (Lebreton et al. 2017). This 44 debris can persist for centuries in the marine environment. Floating plastics in marine 45 environments have different sizes: large debris (macroplastics) and smaller particles, which are 46 further classified into mesoplastics (< 20 mm), microplastics (< 5 mm) and nanoplastics (< 1 47 mm) (Barnes et al. 2009; Hanke et al. 2013; Provencher et al. 2017). The Indian Ocean is 48 particularly affected by the accumulation of plastic due to an oceanic gyre that concentrates 49 debris (van der Mheen et al. 2019; Chenillat et al. 2021). 50

51 Plastic pervades aquaculture settings. In open water aquaculture, free-floating plastic debris from the ocean can enter the cages, and the aquaculture infrastructure itself is mainly composed 52 53 of plastic (e.g. buoys, pipes, nylon nets). Over time, this plastic tends to degrade under the action of ultraviolet radiation, waves and wind, releasing small particles into seawater (Song et 54 55 al. 2017). These particles can subsequently be ingested by marine organisms, including reared animals (reviewed in Barboza et al. 2018; Walkinshaw et al. 2020; Bowley et al. 2021; Chen et 56 57 al. 2021). A number of consequences of plastic ingestion by aquatic animals have been described: for instance, obstruction, intoxication, or physiological and behavior modifications, 58 59 all of which can lead to death (Oliveira et al. 2013; Kershaw 2015; Law 2017; Yin et al. 2018; de Sá et al. 2018). 60

In addition to their direct mechanical action, plastics can also adsorb molecules and serve as 61 potential sources of chemical contaminants of their own composition (Andrady et al. 2009) or 62 from the environment (Mato et al. 2001; Rios et al. 2010). Additionally, the formation and 63 accumulation of microbial biofilms on plastic polymer surfaces, facilitated through various 64 physical and chemical interactions, including van der Waals forces, electrostatic interactions, 65 and hydrophobic interactions, can contribute to the persistence and spread of harmful animal 66 pathogens (Oberbeckmann et al. 2016; Frère et al. 2018; Martínez-Campos et al. 2022) and 67 68 human pathogens (Zettler et al. 2013; Delacuvellerie et al. 2022; Lear et al. 2022; Liang et al. 2023). For example, the accumulation of pathogenic Vibrio species on marine plastic debris has 69

been described in several studies (Sun et al. 2020, Silva et al. 2019; Laverty et al. 2020; Bhagwat 70 71 et al. 2021, Pedrotti et al. 2022). Furthermore, biofilms developing on plastics are known to provide a favorable environment for the development of antibiotic resistance (reviewed in Dong 72 73 et al. 2021) due to slow antibiotic penetration, accelerated horizontal transfer, or antibiotic antagonists (Stewart et al. 2001; Mah 2012; Lebeaux et al. 2012; Uruén et al. 2021). This 74 75 situation is of particular concern in aquaculture systems, where, driven by economics, antibiotic 76 use is widespread in order to prevent and control bacterial diseases in farmed species. Misuse and overuse of antibiotics in aquaculture settings can lead to the emergence and spread of 77 78 antibiotic-resistant bacteria (Haya et al. 2001; Boxall et al. 2004), with significant consequences 79 for public health and the environment. Yet the pathogenic risk posed by plastics as fomites of 80 pathogenic bacteria and carriers of antibiotic resistance genes has received little attention to date, and even less in the context of food security. 81

Assessing the extent of macroplastic contamination by pathogens and associated antibiotic 82 resistance in aquaculture is crucial to identify a potential decline in food production due to 83 84 bacterial infections as well as to minimize the health risk to consumers. The objective of this study was to characterize the pathobiome of macroplastics used in fish farm infrastructure 85 ("aquaculture plastics") and its antibiotic resistance using metabarcoding and bacterial isolates. 86 More specifically, our first aim was to explore the relative abundance and diversity of 87 macroplastic-associated bacterial communities in aquaculture and to determine whether they 88 differ from those in the surrounding water and sediment, in floating macroplastics, and in 89 90 aquaculture fish guts. The second aim was to focus specifically on potential pathogens and their antibiotic resistance genes. The third aim was to investigate the influence of the proximity of a 91 river on pathogen diversity and antimicrobial resistance in the marine environment around the 92 93 farm. Finally, we compared the plastic and fish pathobiomes to determine to what extent these are shared in order to evaluate the role of aquaculture plastics as fomites in fish farms. 94

## 95 Materials and methods

## 96 <u>Sample collection</u>

The sampling for this experiment took place in November 2021 in the vicinity of the Mahébourg
fish farm in Mauritius, which rears red drum (*Sciaenops ocellatus*) in open water cages. Four
sites were sampled: site 1 (20°18'49.4"S, 57°47'03.8"E) and site 2 (20°21'27.1"S, 57°46'54.8"E)
were two fish cages in different locations, site 3 (20°17'13.18" S, 57°46'42.49" E) was the
estuary of Grand River South East that empties near the farm, and site 4 (20°19'20.36" S,
57°48'57.55" E) was the northern channel of the Mauritian lagoon (Suppl. Fig. 1).

103 Bacterial communities from water and sediment were collected in triplicate at each sampling 104 site. Sediment was collected in dives at each site, kept in sterile Falcon tubes and stored at 0°C in a portable icebox until arrival at the laboratory, and then stored at -80°C until DNA 105 extraction. Water samples were collected at 1 m below the surface using 500 mL plastic bottles 106 and stored at 4°C in a portable icebox until arrival at the laboratory. Planktonic microbes were 107 collected on 0.2 µm GTTP filters (Whattman®), and the membranes were then placed in sterile 108 cryotubes at -80°C until further analysis. Metadata such as the temperature, salinity and 109 110 conductivity of the water at the sampling sites were measured and gathered (Suppl. Table 1). Nutrient analyses of the water showed equivalent nitrate and phosphate concentrations in the 111 112 four sampling sites. Nitrites and ammoniac concentration were under the detection threshold 113 (data not shown).

"Floating plastics" (free-floating macroplastic waste or channel buoys) were collected using sterile forceps to avoid contamination when found on the four sites (Table 1). In addition, at the two farm sites, "aquaculture plastics" (plastic parts of the fish cages such as ties, buoys, nets and pipes) were sampled in five replicates (Suppl. Fig. 2). Each macroplastic was rinsed with MilliQ water to remove non-attached microorganisms. A swab (SK-2S swabs, Isohelix, UK) was then used to collect biofilm from the plastic surface (4 cm<sup>2</sup>). Swabs were stored in sterile microtubes at -80°C until DNA extraction.

Fish (n = 8 per site) were collected using nets in the cages (site 1 and 2) by experienced farm staff. Only adult individuals (standard length:  $57 \pm 18$  cm) were collected in order to avoid bacteriome variability due to different ontogenetic stages. Fish were bought to farmers and conserved on ice in coolers for dissection. The content of the last third of the gut (i.e. hindgut) was homogenized and collected to extract the gut bacteriome (Mouchet et al. 2012; Cheutin et

al. 2021). The gut content was stored at -80°C in a 3 mL cryotube until DNA extraction.

# 127 Bacterial culture and phenotypic antimicrobial resistance (AMR) testing

Fish gut content, sediment and water samples were diluted at a ratio of 1/10 in sterile seawater. 128 For plastic samples, swabs were immersed in 10 mL of sterile seawater and vortexed for 60 129 seconds to detach the biofilm from plastic debris. To specifically target PHPB, a 100 µL sample 130 of these solutions was streaked onto Mueller Hinton agar, a non-selective growth medium, and 131 132 incubated for 24h at 37°C in aerobic conditions, to simulate internal conditions of the human body. All colonies were then collected and inoculated in a tube culture medium (Bio-Rad, 133 France). After 24h of incubation at 37°C with a non-hermetic seal, tubes were kept at room 134 135 temperature until isolate analyses in the lab (~one week).

All samples were then seeded on five different solid culture media: non-selective (chocolate 136 137 agar PolyViteX) and selective either for gram negative bacteria (MacConkey agar), gram positive bacteria (Columbia-CNA agar: blood + colistin + nalidixic acid), bacteria resistant to 138 Beta-lactam (chromID<sup>®</sup> ESBL agar: extended spectrum Beta-lactamase) and bacteria resistant 139 to carbapenems (chromID<sup>®</sup> CARBA SMART carbapenemase-selective agar). Samples were 140 then incubated at 37°C for 24h in an atmosphere of 5% CO<sub>2</sub> to isolate anaerobic species on 141 blood + CNA agar and chocolate PolyXiteX agar, and in aerobic conditions for the others. Each 142 phenotypically different colony was then isolated and identified with a Matrix-Assisted Laser 143 Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometer (Bruker Daltonics, 144 Bremen, Germany) following the manufacturer's instruction for identification acceptance. 145

146 Isolated strains were tested against several antibiotics using the Kirby-Bauer disk diffusion method on Mueller Hinton agar. Since all our isolates grew well on Mueller Hinton agar, no 147 148 NaCl was added to the agar for the antibiotic-resistance tests, even for marine bacteria such as Vibrionaceae (Rubin and Tilton 1975; Singleton et al. 1982; Jo et al. 2020). A panel of 149 150 antibiotics among 36 antibiotic disks (i2a, Pérols, France) were tested for each strain (Suppl. Table 2), according to the European Committee on Guidelines for Antimicrobial Susceptibility 151 152 Testing (EUCAST) (2021, version 1.0). Measurement of the inhibition zone diameters was performed on a "Sirscan automatic" zone reader (i2a, Pérols, France). The interpretation of the 153 154 antibiogram results was performed according to EUCAST recommendations (Vong 2021).

The multiple antibiotic resistance (MAR) index is defined as the proportion of resistance to a panel of tested antibiotics. It was calculated for each strain or group of strains, following the method described by Krumperman (1983): the MAR index is equal to a/b, where "a" represents the number of antibiotics to which the strain was resistant, and "b" represents the number of antibiotics to which the strain was exposed.

## 160 DNA extraction and sequencing

Total genomic DNA from 200 mg of homogenized fish gut contents, GTTP filters and swabs (for plastic samples) was extracted using the MagAttract PowerSoil<sup>®</sup> DNA kit according to the manufacturer's instructions (Qiagen, Courtaboeuf, France) with automated processing and the liquid handling system KingFisher FlexTM (ThermoScientific<sup>®</sup>, Waltam, MA, USA). Nucleic acids were eluted in molecular water (Merck MilliporeTM, Burlington, MA, USA) and quantified on a NanoDrop 8000 TM spectrophotometer (ThermoScientific<sup>®</sup>, Wilmington, MA, USA). The DNA extracts were stored at -20°C until further analyses and PCR amplification.

The V4-V5 region of the 16S rDNA gene was targeted with the universal primers 515F-Y (5'-168 GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') 169 (Parada, Needham and Fuhrman 2016) coupled with platform-specific Illumina adaptor 170 sequences on the 5' ends. Each 50 µL PCR reaction was prepared with 25 µL Taq Polymerase 171 Phusion<sup>®</sup> High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs<sup>®</sup>, Inc., 172 Ipswich, MA, USA), 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 2 µL template 173 DNA, 1.5 µL DMSO, and 19.5 µL molecular water. PCR amplifications involved the following 174 protocol: an initial 98°C denaturing step for 30 s followed by 35 cycles of amplification (10 s 175 denaturation at 98°C; 1 min at 60°C annealing; 1.5 min extension at 72°C), and a final extension 176 of 10 min at 72°C. Amplification and primer specificity were verified by electrophoresis on a 177 178 2.0% agarose gel for confirmation of ~450 bp amplicon size. Extraction of blank samples used as DNA extraction controls and standard mock communities (ZymoBIOMICS Microbial 179 180 Community DNA Standards II, Zymo Research) was performed to evaluate the quality of our sample-processing pipeline. Sequencing was performed on Illumina Miseq by GeT-Biopuces 181 (INSA, Toulouse, France). Raw reads were deposited in the NCBI database under Bioproject 182 number PRJNA895209. 183

### 184 <u>Sequence processing</u>

Raw sequencing data was analyzed in R (version 4.1.1), using the dada2 pipeline (Callahan et 185 al. 2016). Briefly, sequences were first trimmed and filtered based on read-quality profiles 186 (maxN = 0; maxEE = [2,2]; truncQ = 2; and truncLen = [250,250]). Amplicon sequence variants 187 (ASVs) were inferred using the dada2 algorithm (Divisive Amplicon Denoising Algorithm) 188 after pooling dereplicated reads from all samples. Then forward and reverse reads were merged 189 and chimeric sequences were removed. The taxonomic classification of ASVs was performed 190 with the naive Bayesian RDP classifier implemented in dada2 and using the SILVA reference 191 192 database nr\_V132. The ASV count table, taxonomy and sequences were organized in a 193 phyloseq object using the phyloseq package (v.1.28.0, McMurdie et al. 2013) in R.

We used a combination of two methods to remove contaminants from our dataset. First, the R package decontam (Davis et al. 2018) was used to identify ASV contaminants from the dataset based on the "prevalence method" of the package. However, some known extraction kit contaminants listed by Salter et al. (2014), such as ASVs from the genera *Bradyrhizobium* and *Cupriavidus*, remained in our dataset. They were manually removed from the final dataset. Overall, 293 taxa corresponding to 8% of the total reads were removed.

200 In order to identify potential human pathogenic bacteria (PHPB) in our pathobiome dataset, all 201 ASV sequences were blasted against a homemade full-length 16S rRNA gene database derived 202 from the enhanced infectious disease database (EID2, Wardeh et al. 2015) containing bacterial species (i.e. cargos) described to have had interactions with the human species. Our database 203 204 contained 87,405 full-length 16S rRNA from the 878 human bacterial cargos in the EID2 database. Only ASVs matching a 16S rRNA sequence with 100% similarity, 100% coverage 205 206 and on more than 250 bp were included in our pathobiome dataset. The potential pathogenicity of each ASV was subsequently checked in the literature, and only ASVs matching a pathogen 207 described at least once were kept. Of the 110 PHPB ASVs detected in our study, only 11 were 208 209 discarded.

Sample read sums were randomly equalized at 11,900 reads per sample using the phyloseq
package (McMurdie et al. 2013). Three samples were discarded following this process. After
standardization, our final dataset consisted of 1,035,300 sequences belonging to 34,675 ASVs.

213 Statistics workflow

Taxonomic diversity of each microbial community was measured using richness (number of ASVs) and the Shannon diversity index H. Statistical analyses on alpha diversity comparisons

were carried out using a Kruskal-Wallis test followed by a Dunn post-hoc test (p-value corrected by Bonferroni's method). Beta diversity was assessed using Bray–Curtis distance with the vegan package in R (Oksanen et al. 2022), and statistical analyses were performed using permutational analyses of variance (PERMANOVA). Bray–Curtis dissimilarity was shown in a principal coordinate analysis (PCoA) plot.

ASV biomarkers (i.e. differential abundance of ASV between sample types) were identified by

the analysis of bacteriome composition with bias correction (ANCOMBC; p-value corrected by

223 Benjamini–Hochberg method) in the microbiomeMarker package (Cao et al. 2022).

224 Core bacteriomes were identified by examining species abundance distribution (SAD), patterns

of each ASV, and by partitioning the SAD into core and satellite ASVs (Magurran et al. 2003).

226 The index of dispersion for each ASV was calculated as the ratio of the variance to the mean

abundance (VMR) multiplied by the occurrence. This index was used to model whether lineages

follow a Poisson distribution (i.e. stochastic distribution), falling between the 2.5% and 97.5% confidence interval of the chi-squared ( $\chi^2$ ) distribution. Index values lower or higher than 1

230 meant that the ASV was under- or overdispersed compared to the Poisson distribution, such

that it spread uniformly and could be considered a core ASV. Index values close to 1 meant that

the ASV followed a Poisson distribution and corresponded to a satellite ASV.

233

# 234 **<u>Results</u>**

## 235 <u>Composition of the bacterial plastisphere</u>

A total of 34,675 ASVs were retrieved from the 87 samples analyzed in this study. The analysis 236 of these revealed differences in the composition and diversity of bacterial communities in 237 aquaculture plastic, floating plastic, fish guts, and the environment (i.e. water and sediment). 238 Aquaculture plastic (AP) and floating plastic (FP) bacterial communities had significantly 239 lower richness and Shannon diversity values than sediment communities (Dunn post-hoc test, 240 p < 0.01), and higher values than communities associated with the fish digestive tract, this was 241 242 significantly higher in AP (Dunn post-hoc test, p < 0.05; Fig. 1A and 1B). The AP and FP communities were as rich and diverse as planktonic bacterial communities in the water. Sample 243 type (i.e. water, sediment, fish guts, AP or FP) explained a higher proportion of the variance 244 (PERMANOVA, p < 0.001, R2 = 0.273) in bacterial community composition than the sampling 245

site (PERMANOVA, p < 0.001, R2 = 0.0281). In particular, AP and FP bacterial composition 246 turned out to be significantly different from the communities in fish guts or the environment 247 (i.e. water and sediment) as shown in PERMANOVA pairwise comparisons (p < 0.01; Fig. 1C, 248 Suppl. Table 3). Although it is not clear on the two first axes of the PCoA (Fig 1C), the 249 variability of the plastisphere composition was also higher than the variability found in fish gut 250 251 or water samples (betadisper, p < 0.01; Suppl. Fig. 3). A pairwise comparison between AP and FP bacterial communities also highlighted significant differences in their composition (pairwise 252 253 PERMANOVA, p < 0.01; Suppl. Table 3).

254 These differences in bacteriome composition were related to high taxonomic rank differential 255 abundance. Proteobacteria was the most represented phylum among all samples (38.5% of all 256 reads), and was particularly abundant in plastic samples (48.7% for AP and 44.6% for FP) compared to fish gut, sediment and water samples (18.9%, 19.2%, 35.6%, respectively) (Fig. 257 1D). At the ASV level, differential abundance analysis using the ANCOM-BC approach 258 highlighted 556 biomarkers from aquaculture plastics and 88 biomarkers from floating plastics. 259 260 The composition of these markers was similar to the composition of the total communities from plastic samples, with a majority of Proteobacteria, Cyanobacteria and Bacteroidota (49%, 20% 261 and 18% respectively from AP, and 45%, 30% and 16% respectively from FP; Suppl. Fig. 4A). 262 263

## 264 Plastics harbor an abundant and rich pathobiome

Of the ASV sequences retrieved in our samples compared with the sequences of the infectious 265 disease database, 99 were identified as potential human pathogenic bacteria (PHPB), including 266 85 different species and 52 different genera (Suppl. Table 4). These PHPB belonged mainly to 267 the families Listeriaceae and Vibrionaceae (47% and 25% of PHPB reads respectively). In 268 total, PHPB reads represented 4% of all reads in the whole dataset. The percentage of PHPB 269 reads varied according to the sample type. While PHPB reads accounted for less than 1% of 270 reads associated with sediment, water and AP, they represented 2.1% of FP reads and 27% of 271 reads associated with fish digestive tracts (Fig. 2A). We detected 48 ASVs that were part of the 272 273 core pathobiome of our samples, with 6 species appearing in more than 50% of the samples 274 (Cutibacterium avidum, Photobacterium damselae, Staphylococcus haemolyticus, Vibrio parahaemolyticus, Vibrio alginolyticus and Listeria innocua; Fig. 2B). The potential infections 275 276 caused by these species are summarized in Suppl. Table 5. Remarkably, in the core pathobiome, metabarcoding analyses also identified Bacillus anthracis in 8 samples from sediment and 277 278 plastic (AP and even more so in FP).

The pathobiome associated with FP showed higher taxonomical richness than in AP or 279 environmental samples (Dunn post-hoc test, p < 0.01; Fig. 3A). Analyzing PHPB abundance 280 using the Shannon index revealed no significant differences in pathobiome diversity according 281 to sample type. In contrast, PERMANOVA pairwise comparisons showed significant 282 differences in pathobiome community composition according to sample type (Fig. 3C, Suppl. 283 Table 3). In a similar pattern to the whole bacteriome community, AP and FP pathobiome 284 composition differed significantly from fish gut and environmental communities (with an 285 exception between AP and sediment) as shown in PERMANOVA pairwise comparisons (p < 286 287 0.01; Fig. 3C, Suppl. Table 3).

The *Vibrionaceae* family represented a high proportion of PHPB in all sample types, accounting 288 289 for 57% in AP, 36% in FP, 49% in sediment, 60% in water, and 21% in fish gut. Similarly, the *Listeriaceae* family was a ubiquitous PHPB family in all sample types with proportions of 18% 290 291 in AP, 4.2% in FP, 14% in sediment, 7.1% in water, and 54% in fish gut. Staphylococcaceae represented 7.7% of PHPB reads in AP, 4.6% in FP, 6.7% in sediment, 0% in water, and 5.2% 292 293 in fish gut samples. The *Moraxellaceae* family was more represented in plastic samples, with proportions of 3.8% in AP and 15% in FP, and 2.2% in sediment, 0.44% in water, and 2.5% in 294 295 fish samples.

The ANCOM-BC approach highlighted PHPB biomarkers in all sample types (Fig. 4B). *Moraxella osloensis*, found on FP, was the only species identified as characteristic of plastic
substrates.

To investigate the potential transfer of PHPB bacteria between plastics and fish in the farm, we analyzed the shared pathobiome (Fig. 4A). We found that 30 bacterial species (30% of PHPB ASVs) were shared between fish and plastics, including the species with the highest occurrence identified in the core pathobiome: *Listeria innocua, Photobacterium damselae, Vibrio parahaemolyticus, Vibrio alginolyticus, Staphylococcus haemolyticus, Cutibacterium avidum, Moraxella osloensis, Pantoea conspicua, Corynebacterium tuberculostearicum* (Fig. 2B).

Investigating the potential effect of sampling site on PHPB richness and the Shannon index, we found significantly higher values for both indices in sampling site 3 (estuary of the Grand River South East) than in aquaculture sampling sites (Dunn post-hoc test, p < 0.05; Suppl. Fig. 5A and 5B). PERMANOVA results also showed significant differences in the pathobiome composition of site 3 compared to the two aquaculture sites (p < 0.01; Suppl. Fig. 5C, Suppl. Table 6). 311

## 312 Isolated strains and antibiotic resistance

A total of 72 bacterial strains from 27 different species were isolated from all samples, of which 313 48 were gram negative and 24 were gram positive (Suppl. Table 7). The PHPB strains belonged 314 to 19 different species and represented 83% of all isolated strains. Similar to the results from 315 the metabarcoding analyses, isolates were dominated by PHPB from the Vibrionaceae family 316 (47% of all isolated strains), and the same dominant species were identified: Vibrio 317 alginolyticus, Photobacterium damselae and, to a lesser extent, Vibrio parahaemolyticus. Three 318 PHPB strains from the Staphylococcaceae family were also found in isolates (4.2% of all 319 isolated strains), and two PHPB strains of Moraxellaceae were isolated (2.8% of all isolated 320 strains). However, no strains from the *Listeriaceae* family were isolated. Isolated strains from 321 FP samples showed greater diversity in bacterial families compared to other sample types, with 322 seven bacterial families isolated (Aeromonaceae, Bacillaceae, Enterobacteriaceae, 323 324 Moraxellaceae, Nocardiaceae, Pseudomonaceae, Vibrionaceae), while the other sample types had only two or three families (Suppl. Fig. 6). The PHPB strains from the *Moraxellaceae* family 325 326 were found exclusively on plastic samples.

As in the metabarcoding analysis, we investigated the isolates shared between fish and macroplastics (Fig. 4). Three species were found in both types of samples: *Vibrio alginolyticus*, *Photobacterium damselae* and *Staphylococcus epidermidis*. In the metabarcoding results, the two *Vibrionaceae* bacteria were also shared between fish and macroplastic samples, and belonged to the core pathobiome, with occurrence of > 40% in all samples.

332 Antibiograms were carried out on all the strains (n = 72), and the MAR index was calculated. We focused on the antibiotic resistance of *Vibrionaceae* strains (n = 34) as they were 333 preponderant and potentially all pathogenic. There was a high level of resistance to certain 334 antibiotics, notably members of the penicillin family. For instance, the tested strains presented 335 88% resistance to ampicillin (n = 33) and 73% to ticarcillin (n = 44; Suppl. Fig 7). However, 336 337 antibiotics known to be occasionally used in aquaculture (cyclins, quinolones and trimethoprim + sulfonamides) efficiently inhibited the isolates: there was 3% global resistance to the tested 338 quinolones (norfloxacin, ciprofloxacin, ofloxacin and levofloxacin; n = 155) and all tested 339 340 strains were susceptible to trimethoprim + sulfonamides and to tetracycline (respectively n =341 69 and n = 65; Suppl. Fig 7).

A MAR index higher than 0.2 is considered a marker for a high risk of antibiotic contamination 342 (Krumperman 1983; Reverter et al. 2020). In our samples, the mean MAR value was  $0.098 \pm$ 343 0.080, and 88% of all isolated strains had MAR values below 0.2, indicating a low level of 344 multiple antibiotic resistance. Nonetheless, bacterial communities on AP had a higher MAR 345 index (mean for all strains:  $0.15 \pm 0.06$ ; mean for Vibrionaceae strains:  $0.17 \pm 0.06$ ), with 346 significant differences between AP and FP (Fig 5) when considering the MAR index of all 347 isolated strains (Dunn post-hoc test, p < 0.01), and significant differences between AP and non-348 plastic substrates for strains belonging to the Vibrionaceae family (Dunn post-hoc test, p < 349 350 0.05).

Concerning sampling sites, isolates from the estuary area (site 3) had a significantly lower MAR index than other sampling sites. No significant differences were found between the MAR indices of the two aquaculture sites and the lagoon channel (Suppl. Fig. 8).

354

## 355 Discussion

# 356 <u>PHPB and antibiotic-resistant bacteria (ARB) represent a significant component of the</u> 357 <u>aquaculture plastisphere</u>

When immerged in aquatic environments, macroplastics act as unique colonization supports on 358 which microbial biofilms quickly develop. Their composition (large carbon polymers) and 359 360 surface characteristics (hydrophobic) select for specific microorganisms compared to those found in the surrounding environment (Zettler et al. 2013; Dussud et al. 2018; Delacuvellerie 361 et al. 2019). These constitute what has been defined as the "plastisphere" (Zettler et al. 2013). 362 363 Our results were no exception. The bacterial communities associated with aquaculture and floating macroplastics were different to communities found in the surrounding sediment and 364 water, and in fish gut (Fig. 1C). We found a total of 556 ASV biomarkers from aquaculture 365 macroplastics and 88 biomarkers from floating macroplastics (Suppl. Fig. 4A), mainly 366 belonging to the Proteobacteria, Cyanobacteria and Bacteroidia phyla. Other studies have also 367 found these phyla in high proportions on micro-, meso- or macroplastics (Zettler et al. 2013; 368 Oberbeckmann et al. 2016; Dussud et al. 2018; Martínez-Campos et al. 2022). 369

The presence of potential pathogens and/or ARB on plastic substrates has been described in a number of studies (Zettler et al. 2013; Zhang et al. 2020; Kesy et al. 2021; Rasool et al. 2021; Lear et al. 2022; Delacuvellerie et al. 2022; Liang et al. 2023), but our study aimed to address

a gap by investigating their contribution to the whole plastisphere. In metabarcoding analyses, 373 374 we identified 75 PHPB species in aquaculture and floating plastic samples (Suppl. Table 4). The vast majority of the bacterial strains isolated from plastics, mainly dominated by PHPB 375 376 from the Vibrionaceae family, displayed a high level of resistance to antibiotics from the penicillin family. The proportion of PHPB on plastic samples ranged from 0.017% to 7%, with 377 an average of 2.1% on FP and 0.6% on AP (Fig. 2A). Two previous studies have tried to 378 quantify the proportion of pathogenic bacteria in their samples. Using a custom-made 16S 379 380 rDNA gene database, Hou et al. (2021) looked at potentially pathogenic communities 381 developing on incubated microplastics in a mariculture cage and found that these represented on average 0.81% of the whole bacterial plastisphere. Basili et al. (2020) mainly focused on 382 383 fecal indicators on macroplastics collected in coastal sites, and found relatively low abundance of these bacterial indicators, ranging from 0% to 5.1% in the sampled plastisphere. These 384 385 proportions are consistent with our results on plastics (Fig. 2A), although our approach was more conservative than Basili's (based on pathogen taxonomic nomenclature) and Hou's (based 386 387 not only on PHPB but also on fish, mammal, invertebrate and plant potential bacterial pathogens). 388

A critical issue is to determine whether macroplastics (AP and FP) provide a favorable matrix 389 390 for both PHPB and ARB compared to other environmental matrices, acting as potential carriers of infectious diseases and spreading antibiotic resistance from the aquaculture environment to 391 392 fish stock and, ultimately, to humans. This question has been explored mainly on microplastics; 393 several studies have shown a selective enrichment of PHPB and ARB on microplastics compared to inorganic supports or matrices (Kirstein et al. 2016; Frère et al. 2018; Wu et al. 394 395 2019; Junaid et al. 2022; Yu et al. 2022). Zhang et al. (2020) found ARB abundance to be 100 396 to 5000 times higher in the microplastisphere than in the surrounding environment. In addition, 397 the exchange of antibiotic resistance genes (ARGs) between bacterial cells seems to be enhanced in the microplastisphere, as plasmid transfer frequency can be three orders of 398 399 magnitude higher than in free-living microbial communities (Arias-Andres et al. 2018). However, other studies have shown no enrichment of PHPB in the microplastisphere compared 400 401 to control surfaces (glass, wood or cellulose) or particle-attached fraction (Kesy et al. 2019; Hou et al. 2021). Interestingly, we found that aquaculture and floating plastics were enriched 402 403 in PHPB compared to sediment and water samples, with significant differences between FP and 404 sediment (Fig. 2A). In addition, we found higher MAR indices for bacteria sampled on AP 405 compared to non-plastic samples (water, sediment and fish guts), especially concerning the 406 *Vibrionaceae* family (Fig. 5). Aquaculture macroplastics seem to be enriched in ARB compared 407 to environmental samples. Nevertheless, this must be put into perspective as the MAR indices 408 found in this study  $(0.15 \pm 0.06)$  were low compared to the average MAR index of aquaculture-409 related bacteria found in other countries around the Indian Ocean (0.19 in South Africa, 0.35 in 410 Indonesia and 0.36 in India and Sri Lanka; Reverter et al. 2020). This result could be explained 411 by very low antibiotic pressure in this environment.

412 While it is important to note that the molecular detection of PHPB does not prove their pathogenicity, nor their phenotypic resistance to antibiotics, the results of this study suggest 413 that the large amount of plastics typically used in aquaculture infrastructure and the presence 414 of external (non-aquaculture) plastics polluting the farm environment can represent a source of 415 PHPB and ARB in a fish farm. The potential health risks for aquaculture stock and humans 416 caused by these enriched bacteria should not be underestimated, as these plastics may act as 417 dissemination vehicles (fomites) for the spread of infectious diseases and antibiotic 418 419 resistance.

# 420 <u>Aquaculture macroplastics are reservoirs of cultivable ARB that are potentially pathogenic for</u> 421 humans and fish

Metabarcoding data revealed the high variety of PHPB detected on aquaculture and floating 422 423 plastics; the families most commonly associated with potentially harmful bacteria were Vibrionaceae (for AP 0.34% and for FP 0.78% of the whole community), Moraxellaceae 424 425 (0.02% and 0.31%), Listeriaceae (0.11% and 0.09%) and Staphylococcaceae (0.05% and 426 0.1%). Except for the family *Moraxellaceae*, the three other PHPB families are commonly found on the marine plastisphere (reviewed in Junaid et al. 2022), with similar relative 427 428 abundance (Kesy et al. 2019; 2021). Considering the 0.1% threshold generally used to separate the rare bacterial biosphere from more abundant microorganisms (Pedrós-Alió 2012), many of 429 430 these PHPB families represented a significant component of the plastisphere of our samples 431 (Fig. 2A). However, the detection alone of PHPB on plastics is not enough to classify them as 432 environmental reservoirs. A crucial criterion is whether the plastics can support the survival of these PHPB. 433

Of all the plastic PHPB detected in this study, members of the *Vibrionaceae* family were the
most abundant both in metabarcoding and isolate data. More than any other clade, this family
has attracted a lot of attention in literature on the plastisphere (reviewed in Junaid et al. 2022),
particularly in aquaculture systems (Lu et al. 2019; Sun et al. 2020). This is because

438 Vibrionaceae are (1) ubiquitous and abundant in marine ecosystems (Haldar 2012; Ina-Salwany et al. 2019), (2) pioneers in the colonization of the plastisphere (Kesy et al. 2021), and (3) 439 capable of causing human illness (Rivas et al. 2013; Mustapha et al. 2013; Schröttner et al. 440 2020; Letchumanan et al. 2019) or mass mortality of animals reared in aquaculture (Austin et 441 al. 2016; Stentiford et al. 2017; Ina-Salwany et al. 2019; Zhang et al. 2020). In our study, three 442 cultivable species were particularly ubiquitous and abundant on plastics: Vibrio 443 parahaemolyticus, Vibrio alginolyticus and Photobacterium damselae (Fig. 2B). All of these 444 445 species can be pathogenic for humans, causing mainly foodborne diseases and opportunistic 446 infections (Nelapati et al. 2012; Mustapha et al. 2013; Rivas et al. 2013; Suppl. Table 5). They 447 can also be pathogenic for fish, causing skin lesions and ulcers (Romalde 2002; Lai et al. 2014; 448 Marudhupandi et al. 2017) and losses in aquaculture systems (Ina-Salwany et al. 2019; Zhang et al. 2020). In line with previous studies (Zhang et al. 2020; Moore et al. 2020), we found that 449 450 Vibrionaceae PHPB were particularly resistant to beta-lactam antibiotics of the penicillin family (88% resistant to ampicillin and 94% resistant to ticarcillin) but susceptible to 451 452 quinolones and trimethoprim + sulfonamides, which are sometimes used in aquaculture. These results indicate that macroplastics, both external to aquaculture infrastructure and those used in 453 454 aquaculture, are a potential reservoir of ARB that may be pathogenic, and could thus represent a danger to aquaculture stock and human health (Sun et al. 2020; Amaral-Zettler et al. 2020). 455

A particular cultivable PHPB drew our attention: Bacillus anthracis, known to be responsible 456 457 for the lethal zoonosis anthrax. It was found in the metabarcoding dataset, and its culture proved 458 that it was still viable and physiologically capable of dividing and growing. This bacterium is normally found in soils and is well known for its long persistence in the environment: it can 459 remain in soils for up to 100 years. Its persistence in aquatic environments is not well described, 460 461 but spores are thought to be able to survive ~20 months in seawater (Sinclair et al. 2008). In humans, there are cutaneous (most common), gastrointestinal or inhalational forms of anthrax, 462 463 according to the pathway of infection. It particularly affects people working with animals or derived products (Anthrax | CDC 2020; Savransky et al. 2020). No cases in fish or fish farmers 464 465 have yet been described to our knowledge. Although its identification with MALDI-TOF is qualified by the manufacturer's instructions as "excellent," this result needs to be confirmed, 466 467 as both the non-pathogenic *Bacillus thuringiensis* and the pathogenic *Bacillus cereus* are very close genetically to Bacillus anthracis, and we cannot completely exclude bias resulting from 468 469 the identification method used (Spencer 2003; Kolstø et al. 2009; Gee et al. 2014; Marston et

al. 2016). The safety rules of the hospital microbiology department where we performed theisolates did not allow antibiograms on *Bacillus anthracis*.

Overall, our results show that macroplastics, both from external sources and those used in
aquaculture facilities, may serve as breeding grounds for a great variety of physiologically
active PHPB strains and may contribute to the emergence and dissemination of antibiotic
resistance.

### 476 Potential transfer of plastic-associated PHPB and ARB to reared fish

The role of plastics as fomites or reservoirs of pathogens and antibiotic resistance genes is 477 478 drawing increasing attention in the scientific community (Goldstein et al. 2014; Viršek et al. 479 2017; Bowley et al. 2021). The persistence of plastic in aquatic environments and its ability to drift from one place to another make it an effective fomite for potentially harmful 480 481 microorganisms. Rivers and estuaries are known to be a major source of plastic debris in coastal 482 environments (Lebreton et al. 2017); these plastics can be enriched in pathogens and antibiotic-483 resistant bacteria from nearby anthropogenic industries and activities (Zhu et al. 2017; Shih et al. 2021). To investigate this, we looked at the influence of the proximity of the estuary of the 484 485 Grand River South East (3 km and 7.8 km from the two studied aquaculture sites) on the presence of PHPB and ARB in the fish farm. The results showed that samples from the estuary 486 487 displayed the highest taxonomical richness and Shannon diversity of PHPB compared to the 488 other sampling sites (Suppl. Fig. 4). Moreover, some PHPB species - for instance, Vibrio parahaemolyticus and Photobacterium damselae – were shared by plastics from the estuary and 489 from the aquaculture sites, but not by plastics from the lagoon channel. Some of the floating 490 plastics sampled in the estuary were drifting, so further analyses to investigate the role these 491 plastics play in coastal environments as fomites, and the risk they represent in the epidemiology 492 of infectious diseases, would be of interest. In contrast, we found that isolated strains from the 493 494 estuary showed significantly lower MAR indices than other sampling sites, suggesting that the 495 proximity of the river does not represent a risk for the development of ARB in the fish farm. In 496 our study, aquaculture sites, and particularly aquaculture plastics, seem to be the main reservoir 497 of ARB (Suppl. Fig. 8).

The degradation of macroplastics enhances the risks associated with plastic debris because micro- and nanoplastics are more easily ingestible, and potential transfers from these particles to marine biota could occur (Lamb et al. 2018). In an ex situ experiment, Rotjan et al. (2019) proved the persistence of ingested bacteria from microplastic biofilm in coral polyps for several weeks. However, this issue has been less studied for PHPB and edible marine animals,
including reared species, although it is known that these animals ingest plastic (Feng et al. 2019;
Priscilla et al. 2019), sometimes in greater quantities than wild animals (Mathalon et al. 2014).

505 Our metabarcoding analyses identified 30 pathogenic species shared by plastics and fish guts. 506 Of these, two species were cultivable: *Vibrio alginolyticus* and *Photobacterium damselae* (Fig. 507 4A and 4B). The virulence of these isolates was not tested, so their ability to cause infections 508 is not known. Virulence depends on the presence of various virulence factors, but also on their 509 expression in specific environmental conditions (Wassenaar et al. 2001; Diard et al. 2017). It 510 can be presumed that as these isolates were viable and cultivable, their potential to cause 511 infection in reared fish or in human consumers cannot be excluded.

We also identified a further 28 PHPB species shared between plastic samples and fish guts that 512 were not cultivable. Bacteria can reach a viable but not cultivable (VBNC) state to survive 513 under stressful conditions (such as living on plastic in saltwater). Bacteria in a VBNC state 514 cannot be detected by standard laboratory methods, but they can recover their viability and 515 potential virulence in appropriate culture conditions (Fakruddin et al. 2013; İzgördü et al. 2022). 516 The recovery of virulence in Vibrionaceae species after a VBNC state has been shown and 517 merits attention due to its potential impact on food safety and the epidemiology of foodborne 518 diseases (Kahla-Nakbi et al. 2007; Wagley 2023). The observation of 30 PHPB species shared 519 520 by macroplastics and fish suggests that there may be a potential transfer of bacteria associated 521 with plastics after ingestion by animals (Fig. 4A). As some of these are pathogens for humans and marine animals and responsible for foodborne diseases, this is a potential animal and human 522 523 health issue. It should be noted that we cannot exclude the possible contamination of fish through the ingestion of bacteria present in the water rather than plastics. However, as we found 524 the abundance of these pathogens in water lower than that on plastics, this route of 525 contamination may be more moderate. Further ex situ controlled experiments would be valuable 526 to confirm this hypothesis and to prove effective transfer (Beloe et al. 2022). 527

528

## 529 <u>Conclusion</u>

530 This study, carried out in a tropical aquaculture context, showed that the pathobiome on plastics 531 differed significantly from that in fish gut or the environment (water and sediment). We conducted bacterial analyses using metabarcoding and isolates, and both confirmed that 532 533 aquaculture plastics and floating plastics were enriched in PHPB and had a higher MAR index compared to environmental samples. These results highlight the risk that plastics could 534 represent as fomites and reservoirs of potential pathogens and antibiotic resistance in 535 aquaculture systems. The findings also showed that a sizable proportion of pathogen species 536 were shared between fish and plastics (both external and internal to aquaculture infrastructure) 537 (30% of the PHPB ASVs), supporting the hypothesis of a potential risk of pathogen transfer 538 539 from plastics to animals. Further studies would be of interest to test the effectiveness of these transfers in controlled experiments of plastic and bacterial ingestion by fish. 540

541

## 542 List of abbreviations

ARB: Antibiotic-resistant bacteria; AP: Aquaculture plastic; FP: Floating plastic; PHPB:
Potential human pathogenic bacteria; MAR: Multiple antibiotic resistance

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- 564

# 565 Availability of data and materials

566 The online version contains supplementary material and is available at xxx

# 567 <u>Authors' contributions</u>

- TB, JCA and ER obtained the funding for this study. JCA and ER designed the study, collected 568 569 the samples and revised the manuscript. JN contributed to the design of the study, collected the samples, performed the biometrics and all the analyses, performed the statistics analyses and 570 571 wrote the manuscript. CRO created the PHPB 16 rRNA gene database. SG supervised some analyses (cultures and antibiograms). SD contributed to the collection of the samples, and 572 573 performed some cultures and antibiograms. SB welcomed the team to the farm, provided the farm boats and laboratories, and contributed to all the sampling. YB contributed to the 574 575 collection of the samples and to the biometrics analysis. All authors approved the manuscript before submission. 576
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- 580 (YB).

# 581 Ethics approval and consent to participate

Animal handling was performed respecting ethical animal welfare guidelines; the number of
fish sampled was limited to the strictly necessary. Informed consent was obtained from all
individual participants included in the study.

# 585 Consent for publication

586 Not applicable.

# 587 Competing of interest

The authors declare no competing interests that could have appeared to influence the workreported in this paper.

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Figure 1: Composition, alpha and beta diversity of the bacterial community. A: taxonomical richness. B: Shannon diversity. A and B: Dunn test between sample origins, \*\*\*: p<0.001, \*\*: p<0.01, \*: p<0.05. C: Non metric multidimensional scaling (NMDS) ordination of the Bray-Curtis dissimilarities in microbial communities according to the sample type. D: Treemap representing the relative abundance of the most represented phyla (in dark grey) and families (in white) of the microbial community in each sample (> 5%).



Figure 2: Proportion of pathogenic communities and core pathobiome. A: barplot of the pourcentage of pathogenic reads according to the origin of the sample. Dunn test between sample origins, \*\*\*: p<0.001, \*\*: p<0.01. B: occurrence of pathogenic ASVs among samples plotted against its dispersion index. The dotted line depicts the 2.5% confidence limit of the Chi2 distribution: ASVs located above this line are non-randomly distributed among samples, whereas those bellow the line follow a random Poisson distribution. Piecharts display the relative abundance among sample types of the most occurrent ASVs.



Figure 3: Composition, alpha and beta diversity of the pathobiome. A: taxonomical richness. B: Shannon diversity. A and B: Dunn tests between sample types, \*\*\*: p<0.001, \*\*: p<0.01, ns: non significative. C: PCoA plot with Bray-Curtis distances. D: Treemap representing the relative abundance of most abundant phyla (in dark grey) and families (in white) of the pathogenic microbial community in each sample (> 5%).



Figure 4: Venn diagram of PHPB detected (A) in metabarcoding analyses or (B) in isolates according to sample type. Species common in (A) and (B) are written in bold.



Figure 5: MAR index of all isolated strains and *Vibrionaceae* strains according to the sample type. Boxplot represents the median and quartiles for each sample type. The dotted line depicts the MAR threshold above which resistant bacteria are considered as marker of high antibiotic contamination in the area. Dunn test between sample origins, \*\*: p<0.01, \*: p<0.05.



Supplementary figure 1: Geographical representation of the four sampling sites, in Mauritius. Sites 1 and 2 correspond to aquaculture sites, and sites 3 and 4 correspond respectively to the estuary of the South East Grand River and to the lagoon channel.



Supplementary figure 2: Photo of the four aquaculture plastics sampled. A: buoy located between cages. B: net of the cage. C: pipe structure of the cage. D: tie linking the net and the pipes.



Supplementary figure 3: PCoA plot with Bray-Curtis distances (axes 1 and 4) of the diversity of the bacterial communities according to the sample type.



Supplementary figure 4: Biomarkers of the global plastisphere community and of the pathobiome identified by analysis of composition with bias correction (ANCOM-BC). A: treemap representing the relative abundance of the main phyla (>5%, in dark grey) and families (in white) from AP biomarkers (n = 556) and FP biomarkers (n = 88). B: biomarkers of the pathobiome communities according to the sample type, with their standardised effect sizes (W statistic) estimated via the difference on relative abundance between sample types.



Aquaculture\_1 Aquaculture\_2 Estuary Lagoon channel Aquaculture\_1 Aquaculture\_2 Estuary Lagoon channel



Supplementary figure 5: Alpha and beta diversity of the pathobiome according to the sampling site. A: taxonomical richness. B: Shannon diversity. A and B: Dunn tests between sample origins, \*\*\*: p<0.001, \*: p<0.05. C: PCoA plot with Bray-Curtis distances.



Supplementary figure 6: Taxonomy at the phylum level of the isolated strains according to the sample type.



Supplementary figure 7: Proportion of resistant strains against each tested antibiotic, according to the EUCAST recommendations. R: resistant, S: sensible, I: intermedirary (sensible at high concentrations). Amoxi.clav: amoxicillin + clavulanic acid, Ticar.clav: ticarcillin + clavulanic acid, Pipera.tazo: piperacillin + tazobactam, quinu.dalfo: quinupristine + dalfopristine.



Supplementary figure 8: MAR index of all isolated strains and Vibrionaceae strains according to the sampling site. Boxplot represents the median and quartiles for each sample site. The dotted line depicts the MAR threshold above which resistant bacteria are considered as marker of high anibiotic contamination in the area. Dunn test between sampling sites, \*\*: p<0.01, \*: p<0.05.

Sampling site	Aquaculture site 1	Aquaculture site 1 Aquaculture site 2		Lagoorchannel
Longitude	20°18'49.4" S	20°21'27.1" S	20°17'13.18" S	20°19'20.36" S
Latitude	57°47'03.8" E	57°46'54.8" E	57°46'42.49" E	57°48'57.55" E
Sampling date	12/11/2021	12/11/2021	13/11/2021	13/11/2021
Water temperature (°C)	26.53	27.33	26.83	28.07
Water salinity(psu)	30.45	34.9	17.1684	34.3037
Water conductivity (µS/cm)	46782.8	52795.6	27883.8	51954.3

Supplementary table 1: Environmental data of the sampling sites

Antibiotic	Acinetobacter sp.	Aeromonas sp.	Bacillus sp.	Clostridium sp. / Exiguobacterium sp. / Lysinibacillus sp. / Rhodococcus sp.	Proteus sp.	Pseudomonas sp.	Staphylococcus sp.	Vibrio sp. / Photobacterium sp.
Ampicillin (10 µg)					Х			Х
Amoxicillin + clavulanic acid (20-10 µg)					Х			Х
Ticarcillin (75 µg)	Х				Х	Х		Х
Ticarcillin + clavulanic acid (75-10 µg)	Х				Х	Х		Х
Piperacillin (100 µg)					Х	Х		
"Piperacillin + tazobactam (100-10 µg)					Х	Х		
Cefalexin (30 µg)					Х			Х
Cefoxitin (30 µg)					Х			Х
Cefpodoxime (10 µg)					Х			Х
Cefepime (30 µg)	Х	Х			Х	Х		Х
Ceftazidime (30 µg)		Х			Х	Х		
Cefotaxime (30 µg)					Х			
Fosfomycin (200 µg)					Х	Х		
Vancomycin (5 µg)			Х					
Aztreonam (30 µg)		Х			Х	Х		Х
Meropenem (10 $\mu$ g)	Х		Х		Х	Х		Х
Imipenem (10 µg)	Х				Х	Х		Х
Ertapenem (10 µg)				Х	Х			Х
Gentamicin (10 µg)	Х		Х	Х	Х		Х	Х
Netilmycin (10 µg)								Х
Tobramycin (10 µg)	Х				Х	Х	Х	Х
Erythromycin (15 µg)			Х	Х			Х	
Clindamycin (2 µg)			Х				Х	
Quinupristin + dalfopristin (15 µg)							Х	
Fusidic acid (10 µg)							Х	
Linezolid (10 µg)			Х				Х	
Amikacin (30 µg)	Х				Х	Х		Х
Chloramphenicol (30 µg)					Х			Х
Tetracyclin (30 µg)	Х		Х	Х			Х	Х
Colistin (50 µg)								Х
Trimethoprim + sulfamids (1,25-23,75 µg)	Х	Х	Х	Х	Х		Х	Х
Ofloxacin (5 µg)					Х			Х
Ciprofloxacin (5 µg)	Х	Х	Х	Х	Х	Х		Х
Levofloxacin (5 µg)	Х	Х			Х	Х		Х
Norfloxacin (10 µg)							Х	
Nalidixiq acid (30 µg)								Х
Rifampicin (5 µg)							Х	

	AP-Fish	AP-FP	AP-Sediment	AP-Water	Fish-FP	Fish- Sediment	Fish-Water	FP-Sediment	FP-Water	Sediment- Water
Global	0.127	0.0460	0.107	0.222	0.180	0.262	0.474	0.148	0.331	0.442
communities	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )
Pathobiome	0.150	0.0607	0.0450	0.0610	0.139	0.233	0.234	0.122	0.147	0.0956
communities	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.02</b> )	(p = 0.13)	( <b>p</b> = <b>0.02</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.01</b> )	( <b>p</b> = <b>0.03</b> )	( <b>p</b> = <b>0.01</b> )	(p = 0.23)

Supplementary table 3: Summary of results obtained from PERMANOVA pairwise comparisons between beta-diversity estimates, with associated p-values in parenthesis. Bold p-values point out significant differences.

Supplementary tab	le 4: PHPB sequences and taxonomy									
ASV17246	Mean_abundance_inPlasticSamples 0.0390243902439024	Sd_abundance_inPlasticSamples	Sequence BLAST best match TACGTAGGGCGCGAG MSI 801000015	Kingdom Bacteria	Phylum Actinobacteria	Class Actinobacteria	Order	Family Actinomycetaceae	Genus Actinomyces	Species
ASV30006	0	0	TACGTAGGGCGCAAG ATPE01000577	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Neisseria	gonorrhoeae
ASV2023	0.0115279070256876	0.0114289126226386	TACGTAGGGTACAAGI LPXX01000022	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium	ravenspurgense
ASV524 ASV1110	0.0571437210588154	0.0587081442302322	TACGTAGGGTGCGAG LV535263	Bacteria	Actinobacteria Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	matruchotii tuberculostearicum
ASV2839	0	0	TACGTAGGGTGCGAG X84442	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	striatum
ASV1590	0.00160513643659711	0	TACGTAGGGCGCAAG LV535790	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	paraoxydans
ASV16940 ASV15950	0.00638977635782748	0	TACGTAGGGCGCGAG M59055	Bacteria	Actinobacteria Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium Rothia	schleiteri
ASV15954	0.0677966101694915	0	TACGTAGGGCGCAAG KC768807	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Lysinibacillus	sphaericus
ASV540	0.134726346113621	0.220318193667949	TACGTAGGGTGCGAG X79094	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	chlorophenolicum
ASV6991	0.0120077538859174	0.00109148837243858	TACGTAGGGTCCGAG KY234243	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	bacteremicum
ASV27673 ASV547	0.430176565008026	0	TACGTAGGGTGCGAG 246664	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	terrae
ASV28754	0	0	TACGTAGGGTGCGAG KP010754	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia	beijingensis
ASV124	0.0699241161118568	0.0570011384407591	TACGTAGGGTGCGAG JVDS01000009	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	avidum
ASV1285 ASV10630	0.0174901446662843	0.0112092412916308	TACGTAGGGTGCGAG EI785716	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium	acnes
ASV4831	0.0625	0	TACGTAGGGTGCGAG LT547818	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Saccharopolyspora	rectivirgula
ASV1854	0.110421286031042	0.100970458333511	TACGGAAGGTTCGGG KF280289	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	bivia
ASV2813	0	0	TACGGAAGGTCCGGG LG086091	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	copri
ASV17602	0.186046511627907	0	TACGGAAGGTCCAGG LV556048 TACGGAAGGTCCGGG AB547677	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	corporis
ASV33137	0.08333333333333333	0	TACGGAGGATTCAAGI LT558807	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	onderdonkii
ASV13114	0.0263264343018944	0.00614958368321072	TACGGAGGGTGCAAG KJ806354	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	indologenes
ASV16803 ASV23894	0.0508474576271186	0	TACGGAGGGTGCAAG KU358930 TACGGAGGGTGCAAG KR297230	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae Weeksellaceae	Chryseobacterium	indologenes
ASV27450	0.0625	0	TACGTAGGTGGCAAG NBTM01000001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus	viridans
ASV2780	0	0	TACGTAGGTGGCAAG EU723827	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	megaterium
ASV3745	0.0683400316021617	0.0414052864216921	TACGTAGGTGGCAAG MF062970	Bacteria	Firmicutes	Bacilli Bacilli	Bacillales	Bacillaceae	Bacillus	anthracis
ASV17869	0.00319488817891374	0	TACGTAGGTGGCAAG 51784025	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Pseudomonas	monteilii
ASV895	0.0936735260802562	0.0501589092881768	TACGTAGGTGGCAAG JVBO01000005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Streptococcus	salivarius
ASV6179	0	0	TACGTAGGTGGCAAG AB647330	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium	butyricum
ASV2016 ASV3558	0.0335788746142974	0.0255863544788092	TACGTATGGAGCGAG LV534711 TACGTATGGAGCGAG LRPW01000090	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Finegoldia	magna
ASV26945	0.00746268656716418	0	TACGTAGGTGGCAAG Y13364	Bacteria	Firmicutes	Bacilli	Bacillales	Family XI	Gemella	sanguinis
ASV3373	0.0848250081476919	0.126509417723832	TACGTAGGTGGCAAG KU922148	Bacteria	Firmicutes	Bacilli	Bacillales	Family XII	Staphylococcus	epidermidis
ASV18610 ASV30100	0.0277777777777778	0	TACGTAGGTGGCAAG KT275954	Bacteria	Firmicutes	Bacilli Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus	acidilactici
ASV1	0.1712583831779	0.191884510114332	TACGTAGGTGGCAAG X98527	Bacteria	Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria	innocua
ASV822	0.0035831891499693	0.00183120805084868	TACGTAGGTGGCAAG FR687253	Bacteria	Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria	ivanovii
ASV4414	0.0512067226830009	0.0525353451447666	TACGTAGGGGGCTAG LSQZ01000058	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus	anaerobius
ASV103	0.105676012259031	0.074894087309083	TACGTAGGTGGCAAG NDWY01000001	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	haemolyticus
ASV2327	0.17930086410055	0.179137420999734	TACGTAGGTGGCAAG KT720156	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	pettenkoferi
ASV7317	0.093558282208589	0.06857008067762	TACGTAGGTGGCAAG MUXI01000016	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	saprophyticus
ASV16554 ASV1268	0.0578666547767671	0.0736188506371449 0.0423330254436766	TACGTAGGTGGCAAG KU740175 TACGTAGGTCCCGAGLNCVL01000053	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	mitis
				DALIPUA	FILMICUTES	Bacilli	LACIODACIDAIPS	SUPPORTOUTATE	11	
ASV2956	0	0	TACGTAGGTCCCGAGI NCVK01000046	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	mitis
ASV2956 ASV5890	0 0.113230519480519	0 0.162664038796611	TACGTAGGTCCCGAGI NCVK01000046 TACGTAGGTCCCGAGI KT725398	Bacteria Bacteria	Firmicutes Firmicutes Firmicutes	Bacilli Bacilli	Lactobacillales Lactobacillales	Streptococcaceae Streptococcaceae	Streptococcus Pantoea	mitis agglomerans
ASV2956 ASV5890 ASV7829 ASV12654	0 0.113230519480519 0.0475193534251911 0.0195131951319512	0 0.162664038796611 0.0500766673916112	TACGTAGGTCCCGAGI NCVK01000046 TACGTAGGTCCCGAGI KT725398 TACGTAGGTCCCGAGI LAWM01000014	Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes	Bacilli Bacilli Bacilli Bacilli	Lactobacillales Lactobacillales Lactobacillales Lactobacillales	Streptococcaceae Streptococcaceae Streptococcaceae	Streptococcus Pantoea Streptococcus	mitis agglomerans gordonii
ASV2956 ASV5890 ASV7829 ASV12654 ASV13421	0 0.113230519480519 0.0475193534251911 0.0195121951219512 0.0149224956766198	0 0.162664038796611 0.0500766673916112 0 0.0100231780709363	TACGTAGGTCCCGAGI NCVK01000046 TACGTAGGTCCCGAGI KCVK01000046 TACGTAGGTCCCGAGI LAWM01000014 TACGTAGGTCCCGAGI LX0U01000013 TACGTAGGTCCCGAGI MCQT01000004	Bacteria Bacteria Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes	Bacilli Bacilli Bacilli Bacilli Bacilli Bacilli	Lactobacillales Lactobacillales Lactobacillales Lactobacillales Lactobacillales Lactobacillales	Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae	Streptococcus Pantoea Streptococcus Streptococcus Streptococcus	mitis agglomerans gordonii salivarius agalactiae
ASV2956 ASV5890 ASV7829 ASV12654 ASV13421 ASV18416	0 0.113230519480519 0.0475193534251911 0.0195121951219512 0.0149224956766198 0.0265478424015009	0 0.162664038796611 0.0500766673916112 0 0.0100231780709363 0.016848510546096	TAGGTAGGTCCCGAGI NCCKN0100046 TACGTAGGTCCCGAGI NCVK01000046 TACGTAGGTCCCGAGI LAWM01000014 TACGTAGGTCCCGAGI LX0U01000013 TACGTAGGTCCCGAGI MCQT01000004 TACGTAGGTCCCGAGI KF933808	Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes	Bacilli Bacilli Bacilli Bacilli Bacilli Bacilli Negativicutes	Lactobacillales Lactobacillales Lactobacillales Lactobacillales Lactobacillales Lactobacillales Selenomonadales	Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Veillonellaceae	Streptococcus Pantoea Streptococcus Streptococcus Streptococcus Veillonella	mitis agglomerans gordonii salivarius agalactiae parvula
ASV2956 ASV5890 ASV7829 ASV12654 ASV13421 ASV13421 ASV18416 ASV23784	0 0.113230519480519 0.0475193534251911 0.0195121951219512 0.0149224956766198 0.0256478424015009 0.011363636363634	0 0.162664038796611 0.0500766673916112 0 0.0100231780709363 0.016848510546096 0	TACGTAGGTCCCGAGi NCVX01000046 TACGTAGGTCCCGAGi NT25398 TACGTAGGTCCCGAGi LXV01000014 TACGTAGGTCCCGAGi LX0001000011 TACGTAGGTCCCGAGi LX0001000013 TACGTAGGTCCCGAGi NCQT11000004 TACGTAGGTCGCAGAG KF933808 TACGTAGGTGCAAG KF933808	Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes	Bacilli Bacilli Bacilli Bacilli Bacilli Bacilli Negativicutes Negativicutes	Lactobacillales Lactobacillales Lactobacillales Lactobacillales Lactobacillales Lactobacillales Selenomonadales Selenomonadales	Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Veillonellaceae Veillonellaceae	Streptococcus Pantoea Streptococcus Streptococcus Streptococcus Veillonella Dialister	mitis agglomerans gordonii salivarius agalactiae parvula invisus
ASV2956 ASV5890 ASV7829 ASV12654 ASV13421 ASV13421 ASV23784 ASV23784 ASV2549 ASV2549	0 0.113230519480519 0.0475193334251911 0.0195121951219512 0.0149224956766198 0.0265478424015009 0.011363633636364 0.0702990349069125 0.01180618225337098	0 0.162664038796611 0.0500766673916112 0 0.0100231780709363 0.016848510546096 0 0.0942167523620176 0.010128574224738	TACGTAGGTCCCGAGI NCVK01000046 TACGTAGGTCCCGAGI NT25398 TACGTAGGTCCCGAGI LVN01000014 TACGTAGGTCCCGAGI LX0010100013 TACGTAGGTCCCGAGI LX001000004 TACGTAGGTGCCAGI CH32661 TACGGAGGGTGCAAGI L27M0100016 TACGGAGGGTGCAAGI L27M0100016	Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Proteobacteria Proteobacteria	Bacili Bacili Bacili Bacili Bacili Bacili Negativicutes Negativicutes Gammaproteobacteria Alphanorteobacteria	Lactobaciliales Lactobaciliales Lactobaciliales Lactobaciliales Lactobaciliales Selenomonadales Selenomonadales Aeromonadales	Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Veillonellaceae Veillonellaceae Reierinckiareae	Streptococcus Pantoea Streptococcus Streptococcus Streptococcus Veillonella Dialister Aeromonas Methylobacterium	mitis agglomerans gordonii salivarius agalactiae parvula invisus dhakensis radiiotolerans
ASV2956 ASV5890 ASV7829 ASV12654 ASV13421 ASV13421 ASV13421 ASV23784 ASV23784 ASV2549 ASV293 ASV1872	0 0.112230519480519 0.0475193534251911 0.0195121951219512 0.014922495766139 0.0265478424015009 0.0113636363634 0.0702990349069125 0.0180618225373098 0	0 0.162664038796611 0.0500766673916112 0 0.0100231780709363 0.016848510546096 0 0.0942167523620176 0.0101286574224738 0	TACGTAGGTCCCGAGI NCVK0100046 TACGTAGGTCCCGAGI NTZ5398 TACGTAGGTCCCGAGI LVNW01000014 TACGTAGGTCCCGAGI LV0U0100013 TACGTAGGTCCCGAGI LV0U01000004 TACGTAGGTGCCGAGI VF93808 TACGTAGGTGCGAGI LV23661 TACGGAGGGTGCCAGI LV335779 TACGAAGGGGGCTAGI LV335779 TACGAAGGGGGCTAGI LV335779	Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Proteobacteria Proteobacteria	Bacilli Bacilli Bacilli Bacilli Bacilli Bacilli Bacilli Negativicutes Negativicutes Gammaproteobacteria Alphaproteobacteria	Lactobaciliales Lactobaciliales Lactobaciliales Lactobaciliales Lactobaciliales Selenomonadales Selenomonadales Aeromonadales Rhizobiales	Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Veillonellaceae Veillonellaceae Beijerinckiaceae Beijerinckiaceae	Streptococcus Pantoea Streptococcus Streptococcus Streptococcus Veillonella Dialister Aeromonas Methylobacterium Methylobacterium	mitis agglomerans gordonii salivarius agalactiae parvula invisus dhakensis radiotolerans radiotolerans
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ASV2956 ASV5890 ASV7829 ASV12654 ASV13421 ASV13421 ASV23784 ASV23784 ASV2549 ASV2549 ASV293 ASV2695 ASV4908 ASV1305	0 0.113230519480519 0.047519333425191 0.019521951219512 0.0149224956766198 0.0136636636364 0.0702990349069125 0.0180618225373098 0 0 0.0180618225373098 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0.162664038766611 0.0500766673916112 0 0.0100231780709363 0.010484510556096 0.0942167523620176 0.0101286574224738 0 0.0101286574224738	TACGTAGGTCCCGAGI NCVK01000046 TACGTAGGTCCCGAGI NCVK01000014 TACGTAGGTCCCGAGI LAVM010000114 TACGTAGGTCCCGAGI LAV001000013 TACGTAGGTCCCGAGI LAV001000004 TACGTAGGTGCCAGI CZM01000004 TACGTAGGTGCAAGI LZM01000016 TACGTAGGTGCAAGI LZM01000016 TACGAAGGGGGCTGG LV303779 TACGAAGGGGGCTGG LV303779 TACGAAGGGGGCTGG LV303521 TACGTAGGTGCAGG LV395321 TACGTAGGTGCAAGI LX391531	Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria Bacteria	Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Firmicutes Proteobacteria Proteobacteria Proteobacteria Proteobacteria	Bacilii Bacili	Lactobacillates Lactobacillates Lactobacillates Lactobacillates Lactobacillates Lactobacillates Lactobacillates Selenomonadates Selenomonadates Reizobiates Rhizobiates Betaproteobacteriates Betaproteobacteriates	Streptococaceae Streptococaceae Streptococaceae Streptococaceae Streptococaceae Veillonellaceae Veillonellaceae Beijerinckiaceae Beijerinckiaceae Beijerinckiaceae Burkholderiaceae Burkholderiaceae	Streptococcus Pantoea Streptococcus Streptococcus Streptococcus Dialister Aeromonas Methylobacterium Oligella Massilia	mitis agglomerans gordonii salivarius agalactiae parvula invisus dhakensis radiotolerans radiotolerans urethralis timonae
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Bacteria	Hosts (human and/or fish)	Human saprophyte	Infections in humans
Moraxella osloensis	Human	yes	Endocarditis, meningitis, osteomyelitis, septic arthritis, vaginitis, and bacteremia (Shah, Ruth, et Coffin 2000)
Cutibacterium avidum	Human	yes	Breast infections, skin abscesses, infective endocarditis, and device- related infections (Corvec 2018)
Photobacterium damselae	Human and fish	no	Wound infections, necrotizing fasciitis, sepsis, urinary tract infections, food intoxications (Rivas, Lemos, et Osorio 2013, Schröttner et al. 2020)
Staphylococcus haemolyticus	Human	yes	Meningitis, endocarditis, prosthetic joint infections, bacteremia, septicemia, peritonitis, and otitis (Eltwisy et al. 2022)
Vibrio parahaemolyticus	Human and fish	no	Foodborne disease: gastroenteritis, diarrhea, stomach pains, nausea, fever (Letchumanan et al. 2019)
Vibrio alginolyticus	Human and fish	no	Foodborne disease (gastroenteritis, diarrhea, stomach pains, nausea, fever), conjunctivitis, tissue necrosis, peritonitis (Mustapha, Mustapha, et Nozha 2013)
Listeria innocua	Human	no	Bacteriemia, meningitis (Perrin, Bemer, et Delamare 2003, Favaro et al. 2014)
Supplementar pathogens.	y table 5: Sun	nmary of infect	ions that can be caused by some potential

	Aquaculture 1	Aquaculture	1 Aquaculture 1	Aquaculture 2	Aquaculture 2	Estuary
	Aquaculture 2	_ Estuary	_ Lagoon channel	_ Estuary	_ Lagoon channel	_ Lagoon channel
Global	0.0282	0.0514	0.0461	0.0655	0.542	0.0883
communities	( <b>p</b> = <b>0.042</b> )	( <b>p</b> = <b>0.006</b> )	( <b>p</b> = <b>0.048</b> )	( <b>p</b> = <b>0.006</b> )	( <b>p</b> = <b>0.03</b> )	(p = 0.198)
Pathobiome	0.0373	0.0576	0.0267	0.0116	0.0661	0,0955
communities	( <b>p</b> = <b>0.018</b> )	( <b>p</b> = <b>0.006</b> )	(p = 1)	( <b>p</b> = <b>0.006</b> )	( <b>p</b> = <b>0.030</b> )	(p = 0.108)

Supplementary table 6: Summary of results obtained from PERMANOVA pairwise comparisons between beta-diversity estimates, with associated p-values in parenthesis. Bold p-values point out significant differences.

## Supplementary table 7: MALDI-TOF identification of isolates according to their sampling site and type

Organism	Family	Site	Source
Acinetobacter tandoii	Moraxellaceae		3 Water
Aeromonas hydrophila	Aeromonadaceae		3 Floating plastic
Acinetobacter venetianus	Moraxellaceae		3 Floating plastic
Pseudomonas mendocina	Pseudomonadaceae		3 Floating plastic
Aeromonas veronii	Aeromonadaceae		3 Floating plastic
Aeromonas jandaei	Aeromonadaceae		3 Floating plastic
Bacillus cereus	Bacillaceae		1 Sediment
Bacillus pumilus	Bacillaceae		1 Sediment
Photobacterium damselae	Vibrionaceae		1 Sediment
Proteus hauseri	Enterobacteriaceae		3 Floating plastic
Photobacterium damselae	Vibrionaceae		1 Fish
Bacillus subtilis	Bacillaceae		1 Fish
Vibrio alginolyticus	Vibrionaceae		1 Fish
Vibrio navarrensis	Vibrionaceae		1 Water
Vibrio alginolyticus	Vibrionaceae		1 Water
Rhodococcus pyridinivorans	Nocardiaceae		1 Floating plastic
Pseudomonas resinovorans	Pseudomonadaceae		1 Floating plastic
Pseudomonas mendocina	Pseudomonadaceae		1 Floating plastic
Vibrio alginolyticus	Vibrionaceae		1 Floating plastic
Bacillus anthracis	Bacillaceae		1 Floating plastic
Vibrio alginolyticus	Vibrionaceae		3 Sediment
Lysinibacillus pakistanensis	Bacillaceae		3 Sediment
Clostridium sartagoforme	Clostridiaceae		3 Sediment
Vibrio alginolyticus	Vibrionaceae		1 Floating plastic
Vibrio parahaemolyticus	Vibrionaceae		1 Floating plastic
Photobacterium damselae	Vibrionaceae		1 Fish
Acinetobacter tandoii	Moraxellaceae		3 Water
Vibrio alginolyticus	Vibrionaceae		1 Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae		1 Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae		1 Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae		1 Aquaculture plastic
Lysinibacillus fusiformis	Bacillaceae		3 Sediment
Bacillus pumilus	Bacillaceae		3 Sediment
Bacillus amylequofaciens	Bacillaceae		3 Sediment
Vibrio alginolyticus	Vibrionaceae		1 Fish
Photobacterium damselae	Vibrionaceae		1 Fish
Lysinibacillus fusiformis	Bacillaceae		1 Fish
Bacillus pumilus	Bacillaceae		1 Aquaculture plastic
Acinetobacter Junii	Moraxellaceae		3 Floating plastic
Bacillus pumilus	Bacillaceae		3 Floating plastic
Acinetobacter Junii	Moraxellaceae		3 Floating plastic
Vibrio alginolyticus	Vibrionaceae		4 Floating plastic
			4 Floating plastic
Bacilius amylequoraciens	Bacillaceae		a Sediment
Bacilius pumilus	Bacillaceae		4 Sediment
			4 Sediment
Bacilius pumilus Vibrio alginolyticus	Nibrionaceae		4 Sediment
	Vibrionaceae		4 Water
Phodococcus boggii	Nocardiacoao	•	+ Floating plastic
Nilouococcus iloagii Eviguobacterium ca	Racillaceae		2 Electing plastic
Photohacterium damsalaa	Vibrionaceae		2 Aquaculture plastic
Vibrio alginolyticus	Vibrionaceae		2 Aquaculture plastic 2 Water
Vibrio alginolyticus	Vibrionaceae		2 Aquaculture plastic
	VIDITUCCAC		- Aquucultule plastic

Staphylococcus warneri Staphylococcus epidermidis Staphylococcus epidermidis Photobacterium damselae Vibrio alginolyticus Vibrio alginolyticus Vibrio alginolyticus Vibrio alginolyticus Photobacterium damselae Vibrio alginolyticus **Bacillus** pumilus Acinetobacter tandoii Acinetobacter venetianus Vibrio alginolyticus Vibrio alginolyticus **Bacillus** pumilus **Bacillus** pumilus Vibrio alginolyticus

Staphylococcaceae Staphylococcaceae Staphylococcaceae Vibrionaceae Vibrionaceae Vibrionaceae Vibrionaceae Vibrionaceae Vibrionaceae Vibrionaceae Bacillaceae Moraxellaceae Moraxellaceae Vibrionaceae Vibrionaceae Bacillaceae Bacillaceae Vibrionaceae

1 Fish

- 1 Aquaculture plastic
- 2 Fish
- 2 Fish
- 2 Fish
- 2 Fish
- 2 Fish
- 2 Aquaculture plastic
- 2 Aquaculture plastic
- 2 Aquaculture plastic
- 2 Sediment
- 3 Water
- 3 Floating plastic
- 4 Sediment
- 3 Water
- 1 Aquaculture plastic
- 1 Floating plastic
- 3 Floating plastic