1 **Plastic-associated pathogens in marine environments: a meta-analysis.**

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<u>Highlights</u>

- 11 - The plastisphere displays specific potential human pathogenic bacteria (PHPB)

- 12 - PHPB are more abundant in plastic samples compared to the surrounding water column

- 13 - PHPB are not more abundant nor richer on plastics than on other solid substrates

- 14 - *Vibrionaceae* bacteria are the most abundant PHPB identified in plastic samples

- 15 - Marine substrates are a source of PHPB influencing the plastisphere's composition

<u>Abstract</u>

17 The global accumulation of plastic debris in marine ecosystems is continually increasing.

18 Understanding the interaction between these debris and the bacterial biofilm on their surface is

19 essential, particularly with regard to potential human pathogenic bacteria (PHPB). In this meta-

20 analysis, we re-evaluated 16S rRNA metabarcoding data from 35 plastisphere-related studies,

21 published up to late 2022. We compared the bacteriomes of plastics and other marine substrates,

22 identifying PHPB associated with each type of substrate. PHPB were enriched in the

23 plastisphere compared to the surrounding water but did not appear more abundant nor richer in

24 than in other marine solid substrates. Nevertheless, we described a distinctive PHPB signature

25 associated with plastics, including *Vibrionaceae* bacteria (5 % of the whole plastisphere) and

26 biomarkers such as *Staphylococcus haemolyticus*. The contribution of other marine substrates

27 to plastic PHPB was quantified and we found that shell and wood substrates were potential

28 sources of PHPB for plastics. Our results suggest that both plastics and other marine solid

29 substrates could serve as reservoirs for PHPB. However, plastics convey specific PHPB

30 communities, and due to their ubiquity and persistence in marine ecosystems, plastic debris

31 poses a higher risk as fomites compared to other substrates.

32 **<u>Keywords</u>**

33 Plastisphere, Bacterial community, Biofilm, 16S rRNA gene

1. <u>Introduction</u>

34

35   With on average 500,000 tons of plastics entering marine ecosystems annually (Kaandorp et al.

36   2023), the introduction of plastic into the oceans is now widely recognized as a major

37   environmental problem. The prolonged persistence of plastics has led to their increasing

38   prevalence in marine environments, as highlighted in a recent evaluation by Kaandorp et al.

39   (2023) which estimates the total amount of buoyant marine plastic litter at over 3 million tons.

40   Degradation of marine macroplastics under the action of ultraviolet radiation, waves and wind

41   (Song et al. 2017) leads to the formation of small particles classified into mesoplastics (< 20

42   mm), microplastics (< 5 mm) and nanoplastics (< 1 mm) (Barnes et al. 2009; Hanke et al. 2013;

43   Provencher et al. 2017). The high prevalence and the ubiquity of macro- and microplastics in

44   marine environments raise concerns about the growing likelihood of organisms encountering

45   and ingesting plastics. Plastics are thus a potential threat to marine wildlife and reared species,

46   not only by direct effects such as entanglement or mechanical obstruction during ingestion, but

47   also through indirect effects such as chemical intoxication or biological contamination

48   (Kershaw 2015; Law 2017; de Sá et al. 2018; Senko et al. 2020).

49   Interactions between plastic surface and microorganisms have been studied for more than a

50   decade (Harrison et al. 2011) and has generated a rising interest in the scientific community,

51   with more and more articles addressing this subject (Wright et al. 2021). The biofilm that grows

52   on the plastic surface, called plastisphere, is rich in various microorganisms such as bacteria,

53   viruses, archaea, fungi and algae (Zettler et al. 2013; Amaral-Zettler et al. 2020). Surprisingly,

54   the literature does not always converge to the same conclusions concerning the plastisphere

55   description. Some authors describe a bacterial community specific to the plastics (Kirstein et

56   al. 2018; Kesy et al. 2019; Martínez-Campos et al. 2022; Crisafi et al. 2022; Naudet et al. 2023),

57   while other studies found similar communities between plastic and other substrates

58   (Oberbeckmann et al. 2016; Pinto et al. 2019; Kelly et al. 2022). A recent meta-analysis

59   conducted an analysis on Illumina sequencing data from 35 plastisphere-related articles,

60   including marine (n = 26), freshwater (n = 7), and terrestrial (n = 2) studies. They showed that

61   the substrate has a lower impact than many other environmental or study design parameters,

62   such as light regime, environment (marine, freshwater, terrestrial), the type of experiment (in

63   vitro or in situ, collected or incubated plastics), the primer pair used for amplification, and the

64   extraction method (Wright et al. 2021). They however pointed out the higher abundance of

65   plastic-degrading bacteria in plastics than in control samples (Wright et al. 2021).

66 The plastisphere has garnered significant attention, since it serves as a potential host for
67 pathogens capable of inducing infections in both humans and aquatic animals. Several studies
68 describe an enrichment in pathogenic bacteria (higher abundance and/or richness) on plastics
69 compared to environmental samples (Sun et al. 2020; Liang et al. 2023; Naudet et al. 2023),
70 while others report no discernible differences in pathogen levels across different substrates
71 (Hou et al. 2021; Kesy et al. 2021a; Delacuvellerie et al. 2022). The *Vibrionaceae* family
72 emerges as the most frequently cited potential pathogenic bacterial lineage described in the
73 marine plastisphere (Junaid et al. 2022). Bacteria from this family are ubiquitous in marine
74 environments (Haldar 2012; Ina-Salwany et al. 2019) and play a pivotal role as pioneers of the
75 plastic colonization, being abundant in biofilms (Kesy et al. 2021a). Ultimately, they can cause
76 lethal diseases in aquatic species, leading to substantial economic losses in aquaculture (Austin
77 et al. 2016; Stentiford et al. 2017; Ina-Salwany et al. 2019; Zhang et al. 2020). Additionally,
78 they can pose a threat to human health (Rivas et al. 2013; Mustapha et al. 2013; Letchumanan
79 et al. 2019; Schröttner et al. 2020). The evaluation of the colonization of micro- and
80 macroplastics by bacteria potentially pathogenic for humans (PHPB) is essential to identify this
81 new environmental danger for humans and minimize the health risks linked to the consumption
82 of marine products.

83 In this study, we conducted a comprehensive meta-analysis of 16S rRNA gene metabarcoding
84 data derived from 35 articles exploring the composition of bacterial communities associated to
85 plastics in marine environments. The primary objective is to provide a comprehensive overview
86 of the current understanding of the marine plastisphere, specifically delving into the bacterial
87 pathobiome associated with plastics. Additionally, we aim to compare these communities with
88 those found on other substrates within marine ecosystems. This comparative analysis seeks to
89 address the pivotal question of whether plastics pose a higher sanitary risk both for marine
90 ecosystems and human health compared to other substrates.

2. **Materials and methods**

2.1. Search strategy and data selection

This systematic research was conducted in November 2022 using computerized bibliographic databases such as PubMed and Google Scholar. The search included a combination of terms to retrieve articles on plastic microbiomes in marine environment. Articles of interest were limited to the following criteria: (1) published in English language, (2) focused on plastics microbiomes developed in marine ecosystems and natural conditions (*in situ* or *in vitro* with circulating seawater), (3) used metabarcoding analyses. This search resulted in 54 studies. 16S raw reads from 36 studies were publicly available and extracted for further analyses, or retrieved on special request to the authors. The collected metadata is presented in the suppl. table 1 and 2.

A total of 1,769 samples were analyzed over the 36 selected studies. The samples had been collected from seas and oceans all around the world, with a majority of studies originating from the Mediterranean Sea (27 % of all samples), in the North Sea (16 %) and in the China Sea (16 %) (Fig. 1). The number of samples analyzed in each study ranged from four samples (Crisafi et al. 2022) to 304 samples (Scales et al. 2021; Fig. 2A). Samples mostly consisted of plastic substrates (69 % of the sample dataset; Fig. 2B), compared to environmental substrates (seawater and sediment), artificial substrates (paint, copper and glass), and natural solid substrates present in marine ecosystems (shell, stone and wood/cellulose).

Most of the studies focused exclusively on macroplastics (>20 mm), on microplastics (<5 mm), or on mesoplastics (>5 mm and <20 mm), respectively representing 43%, 35% and 8% of all studies, whilst the remaining 14% sampled plastics on a wide range of size (Fig. 2C). Most of the analyzed studies incubated their samples in a marine environment for a known duration (46 %), while the rest of the studies collected samples from the environment (38 %), or incubated *in vitro* in an open circuit of seawater (16 %; Fig. 2D).

Several methods were used for the extraction and amplification of the samples according to the study. Extractions were made using commercial kits or custom extraction protocols, and all 16S amplifications targeted the V3-V4 regions, using different primers according to the study.

2.2. Sequence processing per study

Raw sequencing data were re-analyzed in R (version 4.2.2), using the dada2 pipeline (Callahan et al. 2016). Sequences were trimmed and filtered based on read quality profiles. Amplicon sequence variants (ASVs) were inferred using the dada2 algorithm (Divisive Amplicon

122 Denoising Algorithm) after pooling dereplicated reads from all samples. Forward and reverse

123 reads were then merged and chimeric sequences were removed. Only the forward reads were

124 used for studies where the quality of the reverse reads did not allow to process the samples.

125   2.3.   Combined processing of all studies

126 All studies were merged using the phyloseq package (McMurdie et al. 2013) into a unique

127 phyloseq object containing the whole ASV count table, taxonomy and sequences. The

128 taxonomic classification of ASVs was performed with the naive Bayesian RDP classifier

129 implemented in dada2 and using the SILVA reference database nr_V132.

130 Reads sums were randomly equalized at 10,000 reads per sample using the phyloseq package

131 (McMurdie et al. 2013). One study was discarded following this process (Jiang et al. 2018).

132 After standardization, our final dataset consisted of 356,404 ASVs from 35 articles.

133 Potentially human pathogenic bacteria (PHPB) were identified using a homemade full length

134 16S rRNA gene database derived from the enhanced infectious disease database (EID2, Wardeh

135 et al. 2015) containing bacterial species (i.e. cargos) reported to have had interactions with the

136 human species. This homemade database contains 87,405 full length 16S rRNA from the 878

137 human bacterial cargos in the EID2 database. All the ASV sequences were blasted against this

138 database, and only ASVs matching a 16S rRNA sequence with 100 % similarity, 100 %

139 coverage and more than 200 bp were included in our pathobiome dataset.

140   2.4.   Statistic workflow

141 The richness (i.e. number of ASVs) and Shannon diversity index H were calculated to assess

142 the taxonomic diversity of each microbial community. Alpha diversity statistical comparisons

143 were carried out using a Kruskal-Wallis test followed by a Dunn post-hoc test (p-value

144 corrected by Bonferroni's method). To assess Beta-diversity of the whole communities, the

145 ASV count table was agglomerated to merge ASVs that have the same taxonomy at the family

146 level, overcoming differences in ASVs sequences due to the primer pairs used. Beta-diversity

147 on the pathobiomes was assessed on ASVs count table merged at the species level. The Bray-

148 Curtis distance was measured on this new ASV table, with the vegan package in R (Oksanen et

149 al. 2022), and shown in a principal coordinate analysis (PCoA) plot. Statistical analyses were

150 performed using permutational analysis of variance (PERMANOVA). Species biomarkers (i.e.

151 differential abundance of species between sample types) in plastic and seawater samples were

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

153 corrected by Benjamini–Hochberg method) in the microbiomeMarker package (Cao et al.
154 2022). The SourceTracker package (Knights et al. 2011) was used to quantify the relative
155 contribution of other marine substrates to the plastic pathobiome, at the species level.

156 The pathobiome phylogenetic tree was constructed by aligning pathogenic species using
157 MAFFT (v. 7) with default settings. A maximum likelihood phylogenetic tree was then built
158 using RAxML (v.1.0.0; Kozlov et al. 2019) and visualized on the online web server iTOL
159 (v.6.8.1; Ciccarelli et al. 2006).

3. <u>Results</u>

3.1. <u>Composition of the marine plastisphere</u>

The total dataset represented 356,404 ASVs. Various factors exerted selection pressure influencing the composition of bacterial communities. These factors include the source study (PERMANOVA, R2 = 0.414), geographical location (PERMANOVA, R2 = 0.169), primer pair used (PERMANOVA, R2 = 0.127), and substrate type (PERMANOVA, $p < 0.001$, R2 = 0.104; Suppl. Fig. 1A and 1B). While no significant pairwise differences were observed between studies (pairwise PERMANOVA, $p > 0.05$, Suppl. table 3A), significant differences were noted between different substrate types, geographical locations, and primer pairs used (pairwise PERMANOVA, $p < 0.05$, Suppl. table 3B, 3C, 3D).

The plastisphere significantly differed from bacterial communities associated to seawater and other marine solid substrates as shown in PERMANOVA pairwise comparison ($p < 0.05$, Suppl. table 3C, Suppl. Fig. 1A). *Proteobacteria* was the main phylum identified in plastic samples, followed by *Bacteroidota*, respectively representing 52 % and 18 % of the whole plastic community. The *Vibrionaceae* family represented 5 % of the bacterial community in plastic samples, while it represented less than 1 % in stone, seawater, sediment, copper, glass, and paint samples, but 9 % in the wood/cellulose samples and 29 % of the shell community (Suppl. Fig.2).

3.2. <u>The plastisphere includes potential human pathogenic bacteria</u>

A total of 2,876 ASVs were identified as potential human pathogen bacteria (PHPB) in our total dataset, originating from 456 different species (and 165 different genera), representing 0.8 % of the total number of ASVs analyzed and 4 % of the whole dataset reads.

The proportion of PHPB reads per sample varied significantly according to the substrate type (Fig. 3). Plastics displayed a significantly higher percentage of PHPB reads (5.8 ± 13.3 %) than seawater samples (1.9 ± 8.6 %). With a mean of 7.3 ± 3.7 % of PHPB reads per sample, shell samples had a significantly higher proportion of PHPB reads than copper, glass, plastic, seawater and sediment samples. In contrast, sediment samples showed the lowest proportion of PHPB reads (0.09 ± 0.15 %; Fig 3).

No significant differences were found between the PHPB richness and the Shannon indices of plastic and seawater samples (Fig. 4A and 4B). Plastic community displays a significantly higher PHBP richness and diversity than sediment, and a lower PHBP richness and diversity

than stone, but no significant differences in PHBP richness and diversity with copper, glass, paint, seawater, shell and wood/cellulose (Fig. 4A and 4B). Surprisingly, while alpha diversity indices of the total community of sediment samples was the highest of all sample types (Suppl. Fig. 3), sediment pathobiomes were significantly less rich than plastics, seawater, glass, paint, shell, stone and wood/cellulose (Dunn post-hoc test, $p < 0.05$, Fig. 4A), and the Shannon index was significantly lower than the one of plastics, seawater, glass, paint, and stone (Dunn post-hoc test, $p < 0.05$, Fig. 4B).

3.3. Shared and specific pathobiome of the marine plastisphere

The phylogenetic tree encompassing all PHPB detected across the various substrates of the dataset revealed that 98% of PHPB species were identified in plastic samples (Fig. 5). Most of the PHPB species found in plastics were also detected in the water column. Indeed, 329 species (72 % of plastic pathobiome species) were shared by plastics and seawater. The other marine solid substrates did not display any specific PHPB species (Fig. 6A). Some bacterial families were widespread, as for instance the *Vibrionaceae* and *Pseudomonadaceae* families that were found in eight out the nine analyzed substrates (Fig. 5). *Vibrionaceae* was the most represented pathogenic family (33 % of the whole PHPB reads), and was particularly abundant in plastics, wood/cellulose and shell samples (35 %, 68 % and 95 % of the identified PHPB reads respectively). The *Vibrionaceae* family also includes the two most abundant PHPB ASVs identified in plastic samples: *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. The *Pseudomonaceae*, *Moraxellaceae* and *Bacteroidaceae* were then the most abundant families in plastic samples (9.1 %, 8.3 %, 5.6 % of plastic PHPB reads).

Some families appeared contrariwise quite specific to plastic and seawater samples as for instance the *Tannerellaceae*, and *Enterococcaceae* families that were detected in plastic, seawater and glass samples. These families were however detected at low levels of abundance in plastic samples (Fig. 5). Overall, 101 PHPB species were specific to plastic samples (22 % of PHPB species identified). The ANCOM-BC approach was used to identify biomarker species from plastics and seawater (Fig. 6B). Five species were found significantly more abundant in plastic samples compared to seawater and were identified as plastic biomarkers: *Streptococcus salivarius, Psychrobacter nivimaris, Cutibacterium avidum, Staphylococcus haemolyticus* and *Vibrio alginolyticus*. Furthermore, pairwise PERMANOVA comparison highlighted significant differences between PHPB communities from all substrates (copper, glass, paint, seawater, sediment, shell, stone, and wood/cellulose) and plastic pathobiome (Suppl. table 3E). The

223 geographical location of the samples also significantly affected the pathobiome composition
224 (PERMANOVA, R2 = 0.114).

225   3.4.   <u>Tracking the origin of the plastisphere pathobiome</u>

226 The marine environment in which plastics are immersed can contain many different important
227 reservoirs and vectors for the transmission of PHPB, but the occurrence and abundance of these
228 PHPB on plastics, and the source of those microbes have not been extensively described. Using
229 data from the different substrates analyzed in this meta-analysis, we found that shell and
230 wood/cellulose were significant sources PHPB for plastics (Fig. 7). Overall, plastics had
231 10 ± 19 % shell signal (i.e.; 10% of plastic PHPB reads came from shell pathobiomes) and
232 13 ± 20 % wood/cellulose signal. Contribution from natural marine solid substrates could be
233 high, as for instance in the study of Kelly et al. (2022) where stone signal reached a mean of
234 73 ± 36 % in plastic samples (n = 15), or in the study of Zhang et al. (2022) where shell signal
235 reached a mean of 64 ± 32 % in plastic samples (n = 73). However, the source of a large
236 proportion of PHPB plastic species remained unknown. Surprisingly, the contribution of
237 sediment and water column microbes to the plastisphere was low (5 ± 7 % and 5 ± 9 %
238 respectively) but was detectable in all the plastic studies (Fig. 7).

4. Discussion

4.1. A better homogeneity between plastisphere study protocols is needed

Studies on plastic bacterial communities are raising a growing interest in the scientific community, with more and more publications each year from all over the world (Wright et al. 2021). Particularly, the interaction between plastic surfaces and potential pathogenic bacteria has raised a great interest, both for micro and macro-plastics (Fig. 2C; Basili et al. 2020; Kesy et al. 2021; Delacuvellerie et al. 2022; Lear et al. 2022; Martínez-Campos et al. 2022; Zhang et al. 2022; Liang et al. 2023). The high number of research developing in this field is accompanied by a high diversity of study designs to describe plastic bacterial communities. This diversity participates in increasing knowledge in this field, but also in making more complex data sharing and comparisons.

In this meta-analysis, the 35 analyzed studies presented results obtained following various study designs, including sampling, DNA extraction and amplification protocols. For instance, the incubation time of plastic in marine environment is variable according to the study, and sometimes even not known when plastics were collected directly from the environment (Fig. 2D). Yet, the plastisphere composition evolves with time and the biofilm development kinetics varies according to the environmental conditions and the substrate, making it complex to compare various sample communities from different incubation times (Pollet et al. 2018; Xu et al. 2019; Hou et al. 2021). Moreover, the substrate controls used for comparisons or the number of samples collected for each substrate varies highly, resulting in a total of 6 samples for copper to 1,224 for plastics analyzed in our meta-analysis (Fig. 2A and 2B). A higher homogeneity in the choice of control substrates, while remaining relevant in the study context, could also help the scientific community to highlight differences in communities found on plastic and other solid substrates. In addition to that, a better homogeneity between studies protocols during extraction and amplification, as for instance the primer pair used could improve comparison of samples across studies. We advise the use of the primer pair 515F-Y/926R described by Parada et al. (2016) which is largely used in microbiome surveys (e.g. earth microbiome project), is very accurate and has a good coverage of extremely common marine taxa. (Metcalf et al. 2022). All study parameters have been proved to influence the bacterial community identified on solid surfaces, sometimes more than substrate itself (Wright et al. 2021). Our results were consistent with this observation. as the study, and more precisely the primer pair used, exerted a stronger effect on bacterial community composition than the substrate type.

271   Besides study protocols, geographical location and environmental factors, such as water salinity
272   or temperature, were also described as major drivers of biofilm bacterial composition
273   (Oberbeckmann et al. 2020; Wright et al. 2021; Lemonnier et al. 2022). Our results made no
274   exception, as we identified the geographical location as a stronger driver of bacterial
275   communities than substrate type.

276   Difficulties in contacting study authors and accessing raw data contributed to the limitation of
277   the dataset used in our meta-analyze. 33 % of the articles that met the inclusion criteria did not
278   provide raw data or metadata on public databases (n = 18), and 14 studies were discarded from
279   analysis for this reason. Reviewers and editors should pay more attention to this criterion during
280   the publication process in order to develop open science.

281   4.2.   <u>The plastisphere harbors a bacterial signature including potentially human</u>
282   <u>pathogenic bacteria</u>

283   In our study, we confirmed previous results concerning a specific signature of the plastic
284   bacterial communities compared to the environment, called "plastisphere". Potentially human
285   pathogenic bacteria (PHPB) have been detected in previous plastisphere researches (Zettler et
286   al. 2013; Y. Zhang et al. 2020; Lear et al. 2022; Liang et al. 2023). In their review, Metcalf et
287   al. (2022) gathered the results from 31 articles on plastisphere in marine and estuarine
288   ecosystems, and counted 15 potentially pathogenic bacterial genera identified in these studies.
289   In our meta-analysis, we gathered and re-analyzed the raw sequencing data from 35 studies,
290   using a homemade full length 16S rRNA PHPB gene database. We could therefore identify a
291   wider community of PHPB on plastic samples, composed of 163 PHPB genera, including the
292   15 listed in Metcalf's meta-analysis.

293   In our results, plastics display an important percentage of PHPB reads in their associated
294   biofilm (5.8 ± 13.3 %) which was significantly higher than in the water column and sediments,
295   but not significantly higher than in other studied substrates (Fig. 3). Similarly, plastic samples
296   showed a higher richness in PHPB reads than sediment, but not higher than other substrates
297   (Fig. 4A). Indeed, shell or stone showed particularly higher abundance (Fig. 3) or higher alpha
298   diversity indices of PHPB reads than plastics (Fig. 4A and 4B). Variation in PHPB abundance
299   between marine solid substrates could be explained by differences in the physical nature of the
300   substrate surface, such as roughness and hydrophobicity (Abdalla et al. 2021; Briand et al.
301   2022), but also the organic or inorganic nature of a substrate that can provide a nutrient source
302   for the heterotrophic bacterial communities developing on it (Tobias-Hünefeldt et al. 2021).

303   Finaly, the load and composition of PHPB in the surrounding environment can also explain

304   such variability. Interestingly, sediments, which are documented as potential reservoirs of

305   pathogens in riverine, estuarine and coastal waters (Perkins 2015; Hassard et al. 2016; B. Zhang

306   et al. 2020; Frank et al. 2024), harbor less PHPB and with lower diversity than plastic samples.

307   These results suggest that plastic may in fact be a more effective potential reservoir of PHPB

308   than sediment. At least from the point of view of abundance, the physical and nutritional

309   properties of biofilm would therefore provide PHPB a better protection against high salinity,

310   starvation and top-down controls (Yin et al. 2019), ensuring higher survival of PHPB than in

311   sediment.

312   Among the ASV biomarkers that were identified as enriched in plastic samples compared to

313   seawater samples, two species are of particular interest knowing their pathogenicity:

314   *Staphylococcus haemolyticus* and *Vibrio alginolyticus* (Fig. 6A). *Staphylococcus haemolyticus*

315   is part of the natural skin microbiota but some strains were identified as responsible of human

316   infections such as bloodstream infections, ocular infections, epididymo-orchitis, urinary tract

317   infections (Eltwisy et al. 2022). *Vibrio alginolyticus* belongs to the usual marine flora and is a

318   common pathogen of marine animals. It causes vibriosis, and becomes more prevalent globally

319   in recent years (Cao et al. 2022). *Vibrio alginolyticus* was classically considered non-pathogenic

320   in humans (except in immune-compromised persons, where infections can be severe and

321   potentially fatal) but is currently qualified as an emerging pathogen (Campanelli et al. 2008).

322   The *Vibrionaceae* family is the most abundant family identified in the whole pathobiome,

323   representing a third of it. It was moreover detected in all tested substrates except for paint

324   (Fig. 5). This result is consistent with previous studies (Junaid et al. 2022; Naudet et al. 2023)

325   and illustrates the ubiquity and the high abundance of these bacteria in marine environments

326   (Sampaio et al. 2022; Namadi et al. 2023). *Vibrio alginolyticus* and *Vibrio parahaemolyticus*

327   were the most abundant ASVs detected on plastic samples (Fig. 5). *Vibrios* are well known

328   pathogens as they can cause human illness (Rivas et al. 2013; Mustapha et al. 2013;

329   Letchumanan et al. 2019; Schröttner et al. 2020) but also fish diseases, leading to economic

330   losses in aquaculture (Austin et al. 2016; Stentiford et al. 2017; Ina-Salwany et al. 2019; Zhang

331   et al. 2020).

332   The high ubiquity of some taxa in marine environments raises the question of the origin and

333   transfer of PHPB between marine substrates, especially marine plastics. The surrounding

334   environment, especially seawater and sediment have been suggested to be a bacterial source for

335   plastic colonization (Jiang et al. 2018; Sun et al. 2020; Bandini et al. 2021). Our results

336 highlighted the potential role of other natural marine substrates in the first colonization of
337 plastics. Depending on the studies, some substrates indeed made a significant contribution to
338 plastic pathobiome communities, as for instance shell (10 ± 19 %), wood (13 ± 20 %) and
339 sediment (5 ± 9 %), as highlighted by the SourceTracker analysis (Fig. 7). Coastal plastic debris
340 may indeed be in contact with other marine solid debris and share a part of their biofilm
341 communities. The contribution of seawater to plastic biofilms was low (5 ± 7 %) but was
342 detected in all studies. The role of seawater may be essential to suspend and convey inherent
343 bacteria and from other substrates to plastics, and to allow inoculation of new colonizers.
344 Nonetheless, a large proportion of the plastisphere pathobiome origin remained unexplained
345 (Fig. 7) suggesting that (i) certain bacteria in the plastisphere come from ancient, terrestrial
346 plastic contamination before the plastic arrived at sea, (ii) that the plastic PHPB come from the
347 rare "invisible ASV biosphere" of different marine substrates (water, sediment, etc), (iii) or that
348 plastic PHPB come from sources not identified in this meta-analysis.

349   4.3.   <u>Marine solid substrates including plastic debris could act as fomite for PHPB</u>
350   <u>communities.</u>

351 The role of plastics as fomites or reservoirs of a specific pathobiome community present on
352 plastic surfaces is drawing increasing attention in the scientific community (Goldstein et al.
353 2014; Viršek et al. 2017; Bowley et al. 2021; Junaid et al. 2022). However, the particularity of
354 plastics as a hub of pathogenic bacteria compared to other solid substrates (wood, glass,
355 stone…) immersed in aquatic ecosystems is questionable (Oberbeckmann et al. 2016; Kelly et
356 al. 2022; Metcalf et al. 2022). In this study, non-plastic marine solid substrates, when compared
357 to plastic samples, exhibited discernible bacterial communities in their biofilms (Suppl. Fig. 1).
358 Additionally, they harbored equally rich and diverse communities of potential human
359 pathogenic bacteria (PHPB) compared to plastic samples (Fig. 4A and 4B). The proportion in
360 PHPB reads measured on these samples were similar to those in plastics, and even higher for
361 some substrates such as shell or stone (Fig. 3). Moreover, as illustrated in the phylogenetic tree
362 in figure 5, PHPB ASVs that were very abundant in plastic samples were also detected in other
363 solid marine substrates to a lesser extent. The few bacterial families that were exclusively
364 identified in plastic samples were detected with low abundance (Fig. 5), and we don't know
365 whether we would have found them on other substrates with a sampling as considerable as for
366 plastics (n = 1224 for plastics, n = 545 for all other substrates). These results highlight that all
367 these marine solid substrates, including plastic debris, could represent potential fomites of
368 PHPB in the marine environment. A better homogeneity in the choice of controls used to

369 compare plastisphere communities to other biofilms could help to discriminate the different

370 roles of these substrates as fomite in marine ecosystems (Metcalf et al. 2022).

371 However, the high mobility of plastic debris in the environment associated to its high

372 persistence in aquatic ecosystems (Turner et al. 2020) make it a better fomite for bacterial

373 communities compared to other solid substrates. Plastic debris were already described as

374 involved in the transfer of harmful microorganisms to marine biota (Lamb et al. 2018). Further

375 studies on the virulence and the effective transfer of pathogenic bacteria from plastic to

376 organisms should be led to assess sanitary risk of plastic as vector of pathogens (Beloe et al.

377 2022). We must also consider that, unlike other natural substrates (i.e. stone, shell), plastic is

378 the product of industrial production and anthropogenic pollution. This last point makes it a

379 specific case in which mankind can take action to reduce the risk posed by plastic as a reservoir

380 of pathogens for humans and marine ecosystems.

381

5. __Conclusion__

383 This meta-analysis compiles information from studies on the plastisphere utilizing 16S rRNA

384 metabarcoding analyses, resulting in an extensive dataset on the marine plastisphere and its

385 related pathobiome. Our analysis underscores the absence of uniformity in the protocols of

386 plastisphere investigations and particularly the variability of primer pairs used which

387 significantly influences the composition of bacterial communities observed and may hinder the

388 discovery of true plastic bacterial biomarkers. The specific composition of plastic marine

389 biofilms, and particularly the high abundance of PHPB of the *Vibrionaceae* family was pointed

390 out by our results. Two ASV biomarkers were identified as enriched in plastic compared to

391 seawater samples, known for their pathogenicity: *Staphylococcus haemolyticus* and *Vibrio*

392 *alginolyticus*. Due to their persistence and mobility, plastics could vehicle and act as fomites

393 for harmful organisms through seas. Further investigations should be made to unveil the

394 sanitary consequences that these plastics could have in marine environment, both for marine

395 species and human consumers and to propose solutions for managing this emerging risk.

396 __List of abbreviations__

397 ASV: Amplicon Sequence Variant; PHPB: Potential human pathogenic bacteria

398 __Acknowledgments__

Availability of data and materials

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

Authors' contributions

407 JN carried out all practical aspects of the study with guidance and technical support from ER,
408 JCA and TB. TB coordinates the 3 projects that surrounded JN's thesis. JN wrote the first draft
409 of the manuscript and all authors contributed substantially to revisions.

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Competing of interest

413 The authors declare no competing interests that could have appeared to influence the work
414 reported in this paper.

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