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# Microplastics in the insular marine environment of the Southwest Indian Ocean carry a microbiome including antimicrobial resistant (AMR) bacteria: A case study from Reunion Island

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## ABSTRACT

The increasing threats to ecosystems and humans from marine plastic pollution require a comprehensive assessment. We present a plastisphere case study from Reunion Island, a remote oceanic island located in the Southwest Indian Ocean, polluted by plastics. We characterized the plastic pollution on the island's coastal waters, described the associated microbiome, explored viable bacterial flora and the presence of antimicrobial resistant (AMR) bacteria. Reunion Island faces plastic pollution with up to 10,000 items/km<sup>2</sup> in coastal water. These plastics host microbiomes dominated by Proteobacteria (80 %), including dominant genera such as *Psychrobacter*, *Photobacterium*, *Pseudoalteromonas* and *Vibrio*. Culturable microbiomes reach 10<sup>7</sup> CFU/g of microplastics, with dominance of *Exiguobacterium* and *Pseudomonas*. Plastics also carry AMR bacteria including  $\beta$ -lactam resistance. Thus, Southwest Indian Ocean islands are facing serious plastic pollution. This pollution requires vigilant monitoring as it harbors a plastisphere including AMR, that threatens pristine ecosystems and potentially human health through the marine food chain.

## 1. Introduction

Marine pollution by plastic wastes and debris is an important source of anthropogenic contamination in the oceans (Thushari and Senevirathna, 2020). This pollution is increasingly seen as a major concern not only for the environment, i.e., contamination of pristine ecosystems with loss of biodiversity, but also for human health through contamination of marine trophic networks (Smith et al., 2018; Wright et al., 2020; Kumar et al., 2021). Plastics, when they enter the marine ecosystem, influence the marine food chain differently in different species due to factors such

as size, shape, polymer composition and chemical additives (Tuuri and Leterme, 2023). Plastics contaminate marine life at many levels, from zooplankton (Cole et al., 2013) to bivalves (Van Cauwenberghe and Janssen, 2014), fishes (Li et al., 2021), reptiles such as turtles (Thibault et al., 2023) to marine mammals (Panti et al., 2019) and, at the end of the marine food chain, humans (Waring et al., 2018). For example, microplastics have been found in most marine organisms along trophic levels in various organs such as intestinal contents, gills or even flesh (Walkinshaw et al., 2020). In addition, the physical and chemical properties of plastic debris allow them to absorb numerous

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contaminants such as chemicals, metals and bacteria (Imran et al., 2019), acting as vectors for these contaminants when ingested by organisms. Therefore, bioaccumulation of plastics for higher trophic organisms has wider impacts on marine food webs with potential impacts on human health (Carbery et al., 2018).

Plastics slowly degrade over time into smaller and smaller particles including those called “microplastics” (particle size between 0.1 and 5 mm) through weathering and physical processes as well as microbial activities (Galloway et al., 2017; Rummel et al., 2017; Jacquin et al., 2019). As a result, plastics remain present in the marine environment over very large time scales and accumulate, with an estimation of microplastic particles in 2014 reaching up to 50 trillion particles and weighing over 200,000 metric tons (van Sebillie et al., 2015). Microplastic contamination of coastal and marine ecosystems reaches up to 140 particles/m<sup>3</sup> in water and 8766 particles/m<sup>3</sup> in sediments (Thushari and Senevirathna, 2020).

These microplastics are durable, often floating substrates with physical and chemical properties that can have negative impacts on entire marine ecosystems over all bathymetric zones (Rochman, 2015; Masry et al., 2021). The physical traits of microplastics negatively impacting the ecosystems and marine organisms are the plastic size, their shape, the polymer composition and the surface chemistry (Lambert et al., 2017). Moreover, the potential harm of microplastics in marine environments is linked to their ability to accumulate contaminants and transport toxic substances and pathogens (Imran et al., 2019; Chouchene et al., 2023). Microplastic substrates offer new habitats and very effective dispersal ways to microbial communities that can attach through the formation of biofilms and drift along oceanic currents (Oberbeckmann and Labrenz, 2020). In addition, microorganisms' communities associated with plastic, the so-called “plastisphere” (Zettler et al., 2013), have been shown to be enriched with pathogenic bacteria, including members of the genus *Vibrio* (Oberbeckmann and Labrenz, 2020). Thus, pathogen-enriched floating microplastics have the potential to disperse over long distances and spread pathogenic bacteria to new marine areas and ecosystems and should therefore be considered as a threat to marine ecosystems as well as to animal and human health (Bowley et al., 2021; Stabnikova et al., 2021).

Among the pathogens underscored on microplastics, many bacterial strains have been found to harbor antimicrobial resistance (AMR) as well as resistance to heavy metals (MRG) (Bowley et al., 2021). These multidrug resistances are correlated with the presence of heavy metals, organic pollutants, and traces of antibiotics in the marine environment, which can adsorb onto plastic biofilms (Imran et al., 2019). These substances are known to promote horizontal gene transfer (HGT) of virulence and resistance *via* mobile genetic elements (MGE) within bacterial communities (Sobecky and Hazen, 2009). Therefore, microplastics and their associated adsorbed chemicals, by promoting horizontal gene transfer in bacteria, contribute to the selection and dispersal of antimicrobial and metal resistance (Arias-Andres et al., 2018; Marathe and Bank, 2022). Finally, microplastics can have a significant impact on the spread of multidrug-resistant (MDR) pathogens, which may represent an additional threat (in terms of dangerousness compared to simple bacterial contamination) to the entire marine-related trophic network, including humans (Wright et al., 2020).

In the Indian Ocean (IO), recent reports highlight plastic accumulation along the coasts from Australia to India, in the Arabian Peninsula, along the coasts of East Africa and of the IO islands (Pattiaratchi et al., 2022). In the open ocean, the authors reported the absence of any rubbish patch in the northern IO, while a significant patch was identified in the southern IO in relation to the South Atlantic Ocean (Pattiaratchi et al., 2022) and the South Pacific Ocean (Maes et al., 2018). According to Pattiaratchi et al. (2022), Reunion Island, an oceanic island located in the southwest part of the IO, is also impacted by this marine plastic pollution. This island, located at the crossroads of southern Africa and the Indian subcontinent, is also strongly affected by the phenomenon of AMR. In the human sphere, Reunion Island is subject to a very high

pressure of importation of MDR and extensively drug-resistant (XDR) pathogens, linked to the population flow in the area (Miltgen et al., 2020; Miltgen et al., 2021; Kamus et al., 2022). However, there is very little data on the environmental spread of these pathogens from human excreta after the discharge of these effluents from the wastewater treatment plants into the ocean (Miltgen et al., 2022). The same is true for microplastics that are subject to human pollution in other territories and that can drift *via* the oceans to Reunion Island.

Thus, the microbial communities existing on the plastic marine pollution that reaches the coasts of Reunion Island could be affected by this AMR phenomenon. Therefore, it is of utmost importance to determine whether the microbial communities colonizing the marine microplastic debris drifting off the coast of Reunion Island host pathogenic bacteria, potentially resistant to several antimicrobials, which should then be considered as a threat to public health.

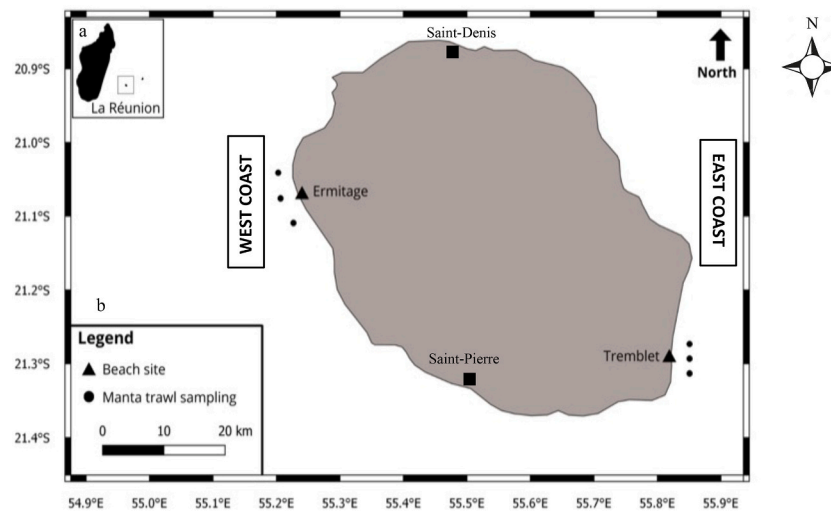
The present study aims at (i) characterizing the microbiome hosted by marine microplastics drifting in the coastal waters of Reunion Island and (ii) addressing the presence of AMR potential pathogens carried by these microplastics. To our best knowledge, this study is the first in the southwest Indian Ocean islands, including physico-chemical, genomic, and microbiological approaches. It integrates public health concerns and local environmental issues with the aim of shedding light on the role of microplastics and the consequences that this new human-induced niche may have, not only on the marine environment and island ecosystems, but also potentially on the entire marine food chain, up to humans, in a One Health approach (Wright et al., 2020).

## 2. Materials and methods

### 2.1. Site description and sampling methods

Reunion Island is located at 55° East 21° South, 700 km east of Madagascar (Fig. 1). The two selected sites (see map in Fig. 1) are distinct in terms of anthropogenic disturbance and oceanic influence: the first site, Livingstone (21°05'02.5"S 55°13'33.6"E), is located on the leeward west coast, at the level of the Ermitage lagoon, in Saint-Gilles municipality, while the second site *i.e.* the Tremblet beach in Saint-Philippe municipality (21°17'38"S 55°48'19"E) is located on the windward east coast. The 1st site is heavily impacted by local anthropogenic activities (Tourrand et al., 2013; Guigue et al., 2015; Lemahieu et al., 2017) while the 2nd site is a newly formed beach, almost untouched by human activity, resulting from a volcanic eruption that occurred in 2007 (Staudacher et al., 2009). The collected samples were, on one hand, the plastics from the coastal seawater (PSW) and the sand beach (PS), and on the other hand, the substrates *i.e.* the coastal sea-water (SW) and the beach sand (S).

Plastic debris were collected from the seawater surface at 200 m from the shoreline using a manta net (mesh size: 500 µm; mouth area: 1.125 m<sup>2</sup>) provided by the non-governmental organization (NGO) “The Ocean Clean Up” (Rotterdam, The Netherlands; <https://theoceancleanup.com>) (Virsek et al., 2016). The speedboat was sailing at 2 knots and the sampling time was 20 min, with sampling days chosen in fair weather. Three transects were set at each site to generate replicates per area (GESAMP, 2019). Between each replicate, the manta net was rinsed externally with a jet of seawater and all plastic particles were collected. Plastic concentration was calculated following Kukulka et al. (2012). Plastic debris collection numbered by sample according to site and substrate are reported in Supplemental Table 1. At the same time, seawater samples collected from the coastal area (3 replicates of 2 L samples per collection) were processed according to the protocol of Hinlo et al. (2017). In parallel to seawater collection, plastic debris were collected from the beach at each site following the protocols of Besley et al. (2016) and using a 1 m × 1 m sampling quadrat. Three 50 × 10 m corridor transects running parallel to the sea were conducted for each site. We report the density of collected plastic wastes in items/km<sup>2</sup>. At the same time, beach sand samples were collected in triplicates



**Fig. 1.** (a) Location map of Reunion island in the southwest Indian Ocean close to Madagascar and Mauritius island; (b) map of the Reunion island including the two major cities and the study site locations.

according to the protocol reported by Almeida et al. (2019). From these plastic sample sorting, sub-samples of 50 microplastics particles (size <5 mm) were randomized (Löder and Gerdtz, 2015) to carry out optimal DNA extraction as suggested by Debeljak et al. (2017). To avoid air contamination, the separation of the microplastics (<5 mm) was carried out in a clean room and under a binocular magnifier under sterile conditions. These randomized subsamples were assembled in triplicates for both DNA and living microbe extractions (see further for the pre-treatment detailed protocol).

## 2.2. Sub-samples pre-treatments

Each microplastic sub-sample of 50 particles was treated according to the protocol of Trachoo (2004) by gentle abrasion to extract DNA and cultivable bacteria while keeping the polymer structure of the plastic intact. For this, 10 g of washed and sterile (see below) silica sand (Sigma-Aldrich, Darmstadt, Germany) was added to a sterile 50 ml Falcon tube (Fischer, Illkirch, France) containing 50 particles of microplastic. Silica sand was treated before use as follows: sand was first washed for 10 min with 2 % hydrochloric acid (Merck, Darmstadt, Germany), then rinsed 3 times with MilliQ water. Silica sand was then washed for 10 min with sodium hypochlorite solution Emplura at final concentration of 2 % (Merck, Darmstadt, Merck, Germany) and rinsed 3 times with MilliQ water. Finally, sand was rinsed once for 10 min with MilliQ water before being autoclaved (120 °C, 20 min, 1 bar). An artificial seawater solution was reconstituted by dissolving 35 g of NaCl (Sigma-Aldrich, Darmstadt, Germany) in 1 L of MilliQ water and then autoclaved (120 °C, 20 min, 1 bar). A volume of 20 mL of reconstituted sterile seawater and 20 g of sterile and washed silica sand were added to each microplastic sample. Similarly, the beach sand samples (20 g in a 50 ml Falcon tube) were supplemented with 20 ml of reconstituted sterile seawater. All samples (microplastics and beach sand) were vortexed for 60 s. The supernatant was collected and divided into two aliquots of 10 ml for DNA extraction and microbiological analysis. Seawater samples collected from the coastal area (3 replicates of 2 L samples per collection) were processed according to the protocol of Hinlo et al. (2017). For each 2 L sample of sea water, there were two separate filtrations of 1 L on a sterile nitrocellulose membrane (0.22 µm): one filter was stored at -20 °C for DNA extraction and the other one underwent resuspension of bacteria by vortexing in 5 ml of reconstituted sterile seawater for microbiological analyses.

## 2.3. DNA extractions, 16S PCR and library preparation

The 10 ml of supernatants from the plastic abrasion or sediment extractions were filtered and sterilized through 0.22 µm nitrocellulose membranes (Merck Millipore, Cork, Ireland). DNA was extracted from all nitrocellulose membranes resulting from the plastic, sediment supernatant and water filtrations, following the protocols of Debeljak et al. (2017). Briefly, the membranes were placed in Qiagen DNeasy Blood and Tissue kit columns (Qiagen GmbH, Hilden, Germany) and DNA was extracted according to the Qiagen manufacturer's instructions. After extraction, DNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific France, Illkirch-Graffenstaden, France). The DNA samples were then sent to Macrogen's Next Generation Sequencing (NGS) platform (Macrogen, Seoul, Korea) for mass DNA sequencing. Library construction and sequencing were performed according to Illumina 16S metagenomic sequencing library protocols to amplify the V3 and V4 region of 16S DNA (Bukin et al., 2019). Two nanograms of genomic DNA were amplified by PCR with 5× reaction buffer, 1 mM dNTP mix, 500 nM of each of the universal F/R PCR primers and Hercules II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycling condition for the 1st PCR was 3 min at 95 °C, and 25 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, followed by a final extension of 5 min at 72 °C. The V3-V4 domain of the 16S rDNA was amplified by PCR using the following primers V3-341F: 5'-CCTACGGGNGGCWGCAG-3', V4-805R: 5'-GACTACHVGGGTAT-TAATCC-3' (Klindworth et al., 2013) with Illumina adaptor overlays. The PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA) and 2 µl of the purified product was PCR amplified for construction of the final library containing the index using the Nextera XT index primer. The cycling condition for the 2nd PCR was the same as the 1st PCR conditions. The PCR product was purified with AMPure beads. The final purified product was then quantified using qPCR according to the qPCR quantification protocol guide (KAPA library quantification kits for Illumina sequencing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany).

## 2.4. NGS analyses

Paired sequencing (2 × 300 bp) was performed using the MiSeq™ platform (Illumina, San Diego, USA). Adapter pruning was performed using the fastp program, adapter sequences were removed and error correction was performed in overlapping sequences (Chen et al., 2018).



The read assembly was performed by assembling pair-end sequences created by sequencing both directions of the library. The program used in this process is \*FLASH (v1.2.11) (Magoc and Salzberg, 2011). Assembled reads shorter than 400 bp or longer than 500 bp were removed. Next, the preprocessing and clustering process was performed according to the protocols of Li et al. (2012): data with sequence errors were removed in order to obtain accurate OTUs. Reads containing ambiguous bases and chimeric sequences were also removed. After this process, clustering was performed based on sequence similarity with a cut-off value of 97 % using CD-HIT-OTU, a comprehensive program based on cd-hit-est. Community diversity and taxonomy were analyzed according to Caporaso et al. (2010) using QIIME (v1.9.0), which is used for OTU analysis and taxonomy information. The main sequence of each OTU was referenced in the NCBI 16S database, and taxonomic information was obtained with BLASTN (v2.4.0).

## 2.5. Microbiological analysis

Successive serial dilutions from 1 to  $10^{-2}$  were prepared from the bacterial supernatant resulting from the plastic/sand abrasion or the seawater 0.22  $\mu\text{m}$  membrane filter resuspension using the reconstituted sterile seawater. The total bacterial flora was counted by inoculating 100  $\mu\text{l}$  of these dilutions onto plate agar of Mueller Hinton + PolyViteX (PVX, BioMérieux, Marcy l'Etoile, France) and Mueller Hinton E (MHE, BioMérieux, Marcy l'Etoile, France) media as previously described (Miltgen et al., 2020; Bhuyan et al., 2023). Once inoculated, the media were incubated at  $35 \pm 2^\circ\text{C}$  for 24 to 72 h, until the microbial colonies appeared visible. The total bacterial flora was quantified and expressed as colony forming units (CFU) per g of plastic or sand or ml of seawater. The following selective media were also used: Columbia NaladixicAcid Agar (CNA, bioMérieux, Marcy l'Etoile, France) for the identification of the Gram-positive bacteria, Drigalski (DRIG, bioMérieux, Marcy l'Etoile, France) for Gram negative bacteria and chromID CPS ELITE (CPSE, bioMérieux, Marcy l'Etoile, France) for a control. For each sample, subcultures were analyzed, and each phenotypically distinct colony was re-isolated on Mueller Hinton agar (MHE) and incubated at  $35 \pm 2^\circ\text{C}$ . After 24 to 72 h, the individual colonies were identified using MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-Of-Flight) mass spectrometry (Bizzini and Greub, 2010). After identification, pathogens were counted on selective media to estimate the relative abundance of each bacterial genus. The antimicrobial susceptibility of each pathogen was assessed by the disk diffusion or gradient strips methods (Miltgen et al., 2020). After 18–24 h, the inhibition diameter around each antibiotic disk or the MIC (minimal inhibitory concentration) were measured and the bacterial/antibiotic pair was categorized susceptible, intermediate or resistant (S/I/R) following the recommendations of The European Committee on Antimicrobial Susceptibility Testing (2020 EUCAST, <https://www.eucast.org>), while the resistance for isolates belonging to *Vibrio* spp. was categorized using the Clinical Laboratory Standards Institute (CLSI <https://clsi.org/>) recommendations.

## 2.6. Microplastic polymer identification

Microplastic particles were retrieved after the sand abrasion as described above. They were characterized by using Fourier Transform InfraRed spectroscopy (FTIR Nicolet i550, Thermo fisher) in ATR (Attenuated Total Reflection) mode, with wavelengths ranging from 400 to  $4000\text{ cm}^{-1}$  (resolution of  $1\text{ cm}^{-1}$ ). The pieces were one by one pictured then pressed between diamond and base (Djaoudi et al., 2022). The diamond was cleaned between each particle analysis. Final infrared spectrums (average of 40 scans) were analyzed using SpectraGryph software and its database. Only correspondences higher than 85 % were validated. The polymer FTIR identification and frequency per sample are reported in Supplemental Table 1.

## 2.7. Microbial community analyses based on OTUs abundance, taxonomy and statistical methods

A full overview of this analytical approach is presented in Supplemental Fig. 1. We used OTUs abundance data resulting from NGS analyses to compare microbial communities between: (i) sampling coasts (East or West coast), (ii) the matrices from which plastics were sampled (sand beach or seawater) and (iii) the sampled materials (plastic, seawater, or sand beach). The sampling DNA sequencing quality was firstly checked with rarefaction curves (Supplemental Fig. 2a, b, c, d) computed with iNEXT online (Chao et al., n.d.). We used a top-down taxonomic approach to explore the differences between microbial communities by first analyzing the full data at the phylum level then focusing only on the most frequent genera belonging to the Proteobacteria phylum (genera representing  $>1\%$  OTUs total abundance: 15 out of the 405 genera identified in the full database, Supplemental Fig. 3). OTU abundances raw data are available as Supplemental Table 2. The composition of microbial communities is presented using (i) barplots of the relative abundance of organisms (cumulated abundances are given in Supplemental Table 2a at phylum level and Supplemental Table 3a for genera belonging to the Proteobacteria phylum level) and (ii) Non-Metric Multidimensional Scaling (NMDS) (based on Bray-Curtis dissimilarities) plots using the “vegan” package for the R software (R Core Team, 2021, v4.0.4). In both graphical representations, data were grouped according to environmental parameters (combination of matrix and material), with NMDS plots showing ellipses for 95 % confidence intervals. Overall differences in bacterial communities' composition were statistically assessed between sample sites, substrates and ecosystems using analysis of similarities (ANOSIM) on the same Bray-Curtis distance matrix (Dixon, 2003). Pairwise differences between groups were statistically assessed using  $\chi^2$  tests and corrected according to the Bonferroni method. Ecological diversity was measured using several indices: specific richness and the Shannon and Simpson diversity indices. Overall differences in ecological diversity between groups were tested using non-parametric Kruskal Wallis test, followed by Duncan's post-hoc tests when more than two groups were compared and non-parametric Wilcoxon Mann-Whitney test if two groups were compared. Venn diagrams were used to illustrate the dissimilarity in composition between groups (Oliveros, 2007).

Linear models were used to compare (i) the abundance of plastic debris collected at the sea surface and on beaches between sites (East vs West) and polymer type and (ii) the abundance of culturable bacterial populations isolated from plastic, water and sand samples from different sites or substrates. Tukey signed-rank tests were further used to evaluate pairwise differences.

## 3. Results

### 3.1. Plastic data

At the sea surface, concentrations of particles were highly variable across samples, ranging from 0 to 7391 items/ $\text{km}^2$  on the west coast and from 3561 to 23,692 items/ $\text{km}^2$  on the east coast (Table 1 and Supplemental Table 1). There was no significant difference between the west and east coasts in terms of item concentrations ( $p\text{-value} > 0.05$ ) (Table 1). Most of the debris (85 %) have been successfully classified by FTIR analysis. Both on the west and east coasts, the most abundant plastic polymers identified were polyethylene (PE), representing 75 % and 84 % of particles respectively; and polypropylene (PP) representing the remaining 25 % and 16 % (Table 1). There was no significant difference between the west and east coast in terms of polymer type ( $p\text{-value} > 0.05$ ). For the beaches, two significant different concentrations of  $0.34 \pm 0.31$  and  $0.022 \pm 0.008$  item/ $\text{m}^2$  were estimated for the west and east coast, respectively. Significant differences between the diversity of polymers on the strandings were found on the west coast, there was 50 % PE, 38 % PP, 9 % polystyrene (PS) and 3 % polyvinyl chloride

**Table 1**

Quantification and characterization of plastic debris collected on East coast and West coast, in seawater or on sand beach. Except for plastic polymer nature, all data are means  $\pm$  stdv of samples (n = 3)/site and substrate collection.

Site	East		West	
Substrate	Seawater	Sand beach	Seawater	Sand beach
Plastic item concentration (mean $\pm$ stdv)	4025 $\pm$ 4760 items/km <sup>2</sup>	0.022 $\pm$ 0.008 items/m <sup>2</sup>	10,693 $\pm$ 11,275 items/km <sup>2</sup>	0.34 $\pm$ 0.31 items/m <sup>2</sup>
Weight of plastic item in mg/item (mean $\pm$ stdv)	1.4 $\pm$ 1.3	31.4 $\pm$ 51.2	2.6 $\pm$ 7.4	56.5 $\pm$ 43.2
Weight of microplastic in mg/sample (mean $\pm$ stdv)	24.7 $\pm$ 0.1	202 $\pm$ 90	46.7 $\pm$ 0.7	1798 $\pm$ 110
Plastic polymer (%)				
Polyethylene	75	70	84	50
Polypropylene	25	29	16	38
Polystyrene	0	1	0	9
Polyvinyl chloride	0	0	0	3

(PVC) while on the east coast, there was 70 % PE, 29 % PP and 1 % PS (Table 1).

### 3.2. Bacterial microbiome analyses based on the 16S rDNA sequencing

#### 3.2.1. Total OTUs diversity and abundance

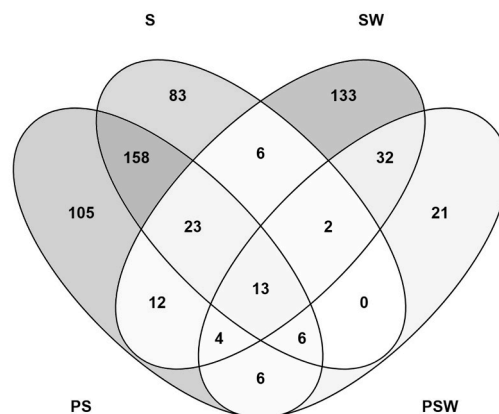
A total of 4,052,436 reads were retained (184,201  $\pm$  22,611 reads per sample on average) after quality filtering and chimera checking, reads abundances ranged from 138,698 to 213,256 for the plastics sampled from seawater of the West Coast (PSWw) and seawater samples from East Coast (SWe), respectively (Supplemental Table 2a, b, c). Read numbers did not differ significantly between plastics and substrates and between coasts (Supplemental Table 2a, b) and no clear grouping was detected when samples were compared pair wisely (Supplemental Table 2c). All rarefaction curves showed an early stationary phase indicating sufficient sequencing depth of the taxa amplified in the microplastic, sand and seawater matrices (Supplemental Fig. 2a, b, c, d). Overall, high-quality sequences were grouped into 1084 OTUs, 877 OTUs were identified from microplastic samples, 747 from the sand samples and 468 from seawater samples. These 1084 OTUs (Supplemental Table 3) were tallied at an overall mean of 21,299  $\pm$  5127 OTUs/sample, with the difference between the East coast and West coast sites being not significant (Supplemental Table 2a,  $p > 0.05$ ), as the difference between plastics sampled in sea water and on plastics sampled in sand (Supplemental Table 2b,  $p > 0.05$ ). However, OTUS were significantly more abundant for plastics than for both of their substrates (seawater or sand), with OTUS being 1.46 times more abundant in plastic DNA samples than in seawater DNA samples, and 1.32 times more abundant for plastics sampled in sand (Supplemental Table 2b,  $p < 0.05$ ). Microplastic debris from seawater shared 28.5 % and 60.7 % of OTUs with seawater in the western and eastern sites, respectively, while microplastics from the sandy beach shared 50.2 % and 61.2 % of OTUs with sand beach in the western and eastern sites, respectively (Fig. 2).

#### 3.2.2. Diversity of OTUs at the phylum level

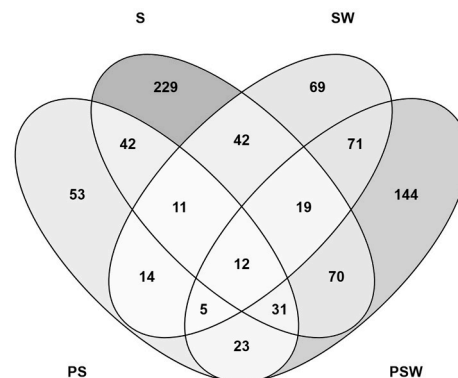
Phylum richness was significantly higher on sand than on water (Table 2). However, there was no significant difference between the study sites (East versus West) nor between plastic versus non-plastic substrates (seawater or sand beach). Shannon and Simpson diversities differed significantly between study sites and between plastic and non-plastic samples (Table 2) but not between sand and water. Shannon and Simpson diversities were higher on the east coast than on the west coast and in non-plastic samples compared to plastic samples (Table 2). The full diversity indexes of the bacterial phylum distribution according to the site and the substrate are presented in Supplemental Table 4a.

An overall number of 19 phyla was identified in both plastics and their substrates samples (Fig. 3a, Supplemental Table 4b). All samples were dominated by Proteobacteria (75 %), Bacteroidetes (11 %), Cyanobacteria (5 %) and Fimicutes (4 %). Actinobacteria, Planctomycetes and Verrucomicrobia were also present across all the samples but at lower levels (<2 %). At the phylum level, bacterial communities associated with plastics harvested in sea water (PSW) stands out from other

a East Coast : (604 OTUs)



b West Coast : (835 OTUs)



**Fig. 2.** Venn diagrams of the operational taxonomic unit (OTUs) distribution showing shared and specific OTUs according to site i.e. East coast (a) or West coast (b) in all sample types i.e. PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand.

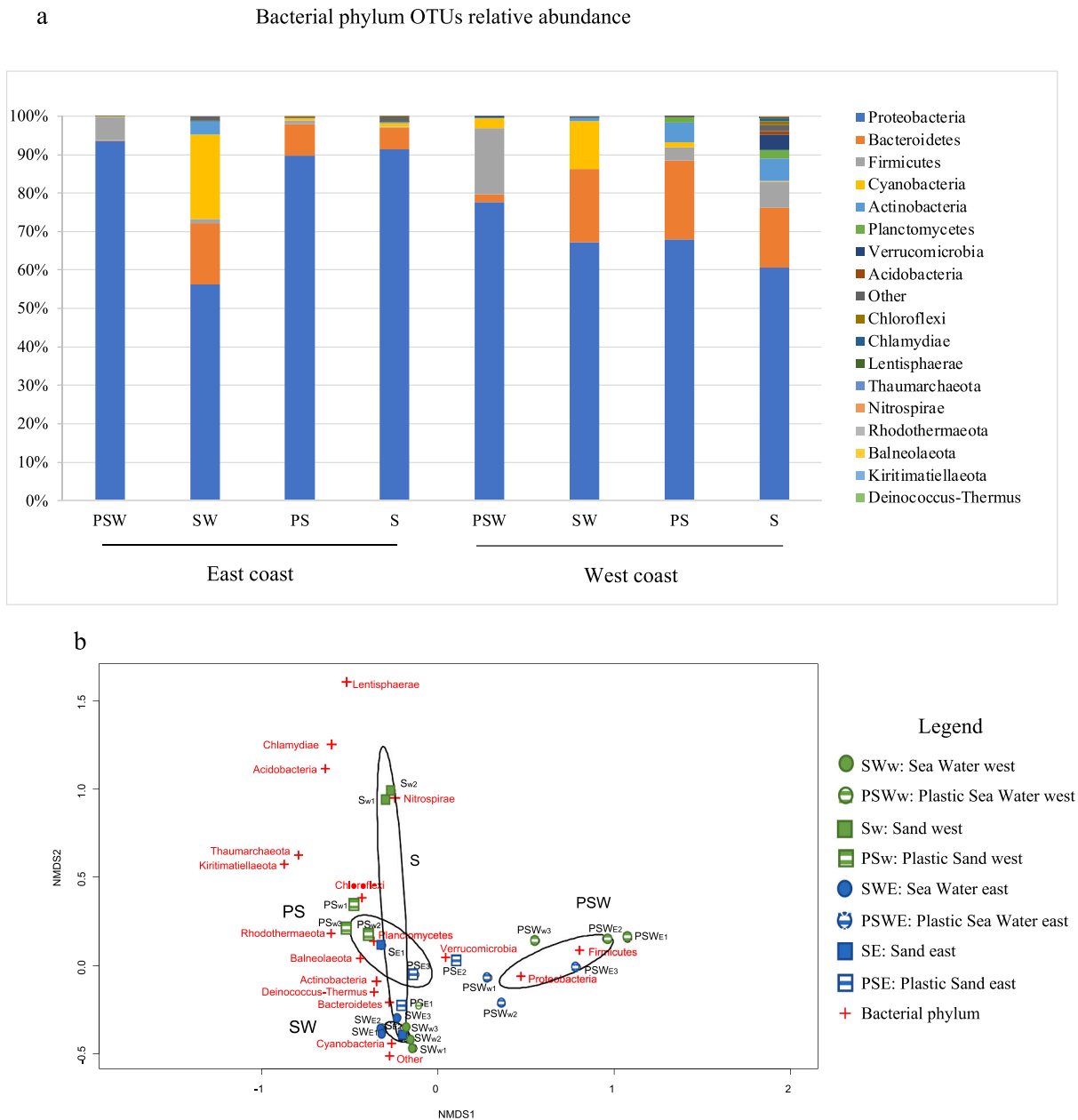
groups (ANOSIM and Chi<sup>2</sup> tests, Fig. 3b and Supplemental Tables 4c and d). Height phyla were detected in seawater and sand but not on plastics: Acidobacteria, Chlamydiae, Gemmatimonadetes, Ignavibacteriae, Kiritimatiellaeota, Lentisphaerae, Spirochaetes and Thaumarchaeota. Phylum communities did not differ between sites (West vs East) and matrices (samples harvested from seawater vs samples harvested from the sand).

#### 3.2.3. Proteobacteria

Proteobacteria were the dominant phylum (75.6 % of the total of

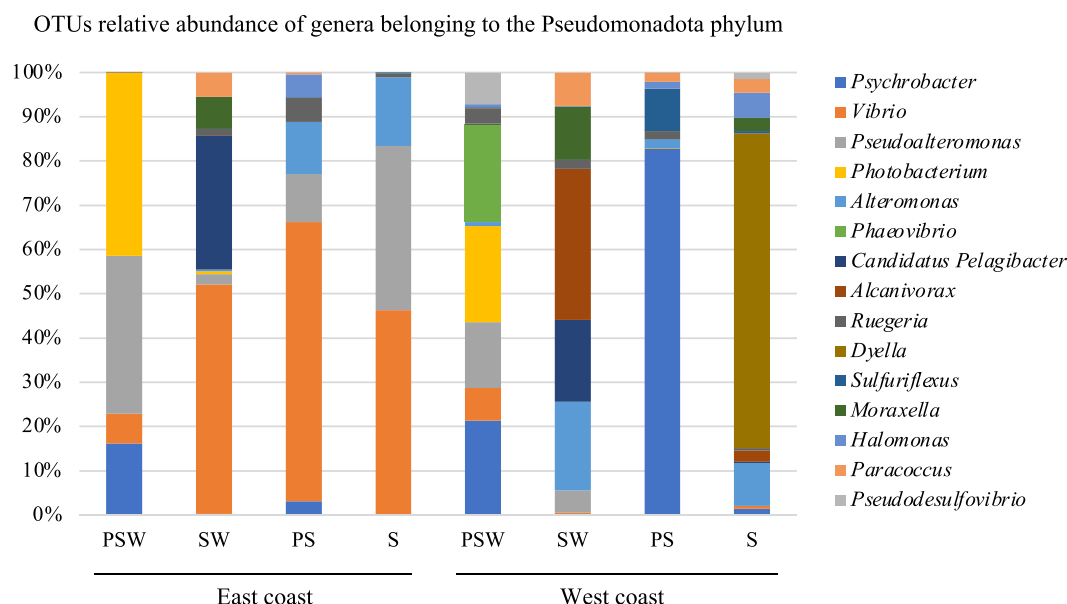
**Table 2**  
Diversity indexes of the marine bacterial phyla and Proteobacteria genera found according to the combination of the site, the substrate, and the presence of plastic. Data are pooled according to three parameters: the study site (East or West), the type of substrate (sea water or sand) and the presence of plastic (plastic or non-plastic substrate i.e., seawater or sand). Data are reported as means  $\pm$  standard errors. Letters indicate significantly different means according to a test of Wilcoxon–Mann–Whitney (at  $p < 0.05$ ). Abbreviations: S: sand; SW: sea water; PSW: plastic from sea water; PS: plastic from sand. Full data of the diversity indexes per site and per substrate are presented in Supplemental Table 3.

Parameters	Groups	Richness		Shannon diversity		Simpson diversity	
		Phylum	Proteobacteria	Phylum	Proteobacteria	Phylum	Proteobacteria
Site	East (S + SW + PSW)	9.8 $\pm$ 3.1 a	8.9 $\pm$ 0.6 a	2.7 $\pm$ 0.8 a	1.05 $\pm$ 0.06 a	2.0 $\pm$ 0.5 a	0.53 $\pm$ 0.03 a
	West (S + SW + PSW)	8.4 $\pm$ 2.7 a	10.3 $\pm$ 0.4 a	1.9 $\pm$ 0.9 b	1.21 $\pm$ 0.11 a	1.6 $\pm$ 0.7 b	0.56 $\pm$ 0.06 a
Substrate	Sand (S + PS)	11.8 $\pm$ 1.8 a	10 $\pm$ 0.6 a	2.4 $\pm$ 1.1 a	1.00 $\pm$ 0.08 a	1.7 $\pm$ 0.6 a	0.48 $\pm$ 0.04 a
	Water (W + PS)	6.9 $\pm$ 1.3 b	9.3 $\pm$ 0.5 a	2.2 $\pm$ 0.8 a	1.24 $\pm$ 0.09 a	1.8 $\pm$ 0.6 a	0.61 $\pm$ 0.04 b
Plastic	Plastic (PSW + PS)	8.6 $\pm$ 2.8 a	9.7 $\pm$ 0.6 a	1.9 $\pm$ 0.6 b	1.03 $\pm$ 0.09 a	1.5 $\pm$ 0.4 b	0.51 $\pm$ 0.05 a
	Non-plastic (SW or S)	9.8 $\pm$ 3.0 a	9.5 $\pm$ 0.6 a	2.8 $\pm$ 1.0 a	1.24 $\pm$ 0.09 a	2.1 $\pm$ 0.7 a	0.60 $\pm$ 0.04 a

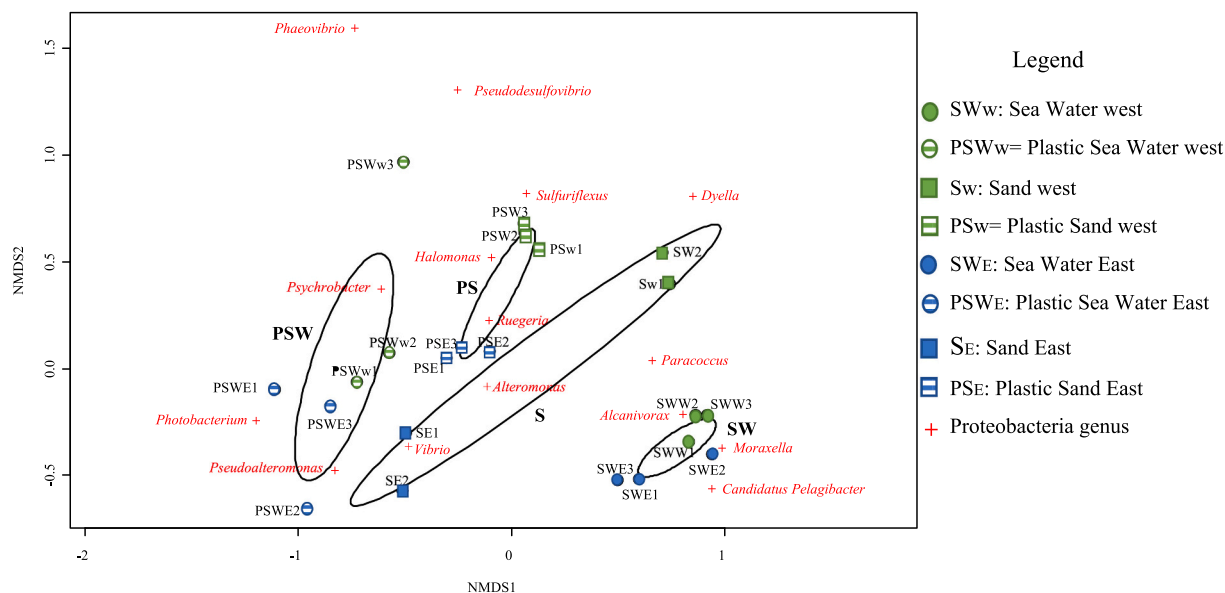


**Fig. 3.** OTUs distribution of the bacterial community according to the analysis at phylum level for the different substrates: PSW: plastics from coastal sea-water; SW: coastal seawater; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of bacterial phyla identified in all samples. (b) Two-dimensional NMDS ordination of bacterial community structure. Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95%) per substrate.

a



b

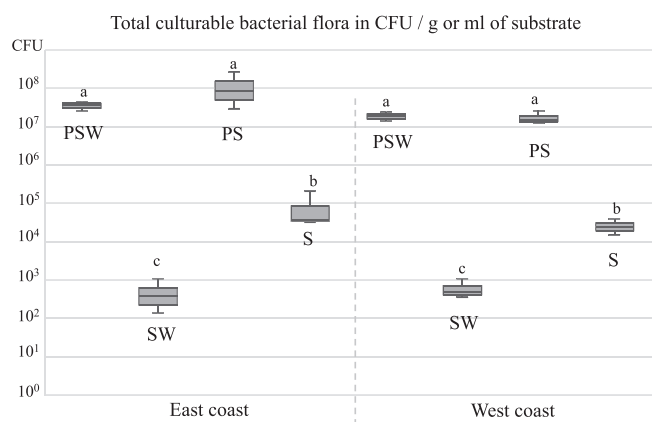


**Fig. 4.** OTUs distribution of the phylum of the Proteobacteria according to the analysis at genera level for the different substrates: PSW: plastics from coastal seawater; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of Proteobacteria genus identified in all samples. (b) Two-dimensional NMDS ordination of Proteobacterial community structure. Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95 %) per substrate.

OTUs) and were further analyzed. To better analyze data at the genus level, we filtered out the least frequent Proteobacteria OTUs (frequency <1 %). 15 out of 405 genera, representing 79.4 % of the total Proteobacteria OTUs were kept (see Supplemental Fig. 3a and Supplemental Table 3 for full results). Genera richness and Shannon diversity did not differ between sites (East vs West) and environmental parameters (sand or seawater), nor between plastic and non-plastic samples (Table 2 and Supplemental Tables 5a, b and c). Simpson diversity did not differ between sites (East versus West) nor between plastic versus non-plastic samples but differed significantly between substrates (sea water versus sand) ( $p < 0.05$ ). Across all samples four genera accumulated >70 % of

the overall OTU abundance: *Psychrobacter* (21.9 % in all samples and 28.8 % in plastics), *Vibrio* (20.2 % in all samples and 17.1 % in plastics), *Pseudoalteromonas* (17.6 % in all samples and 18.1 % in plastics) and *Photobacterium* (14.8 % in all samples and 19.4 % in plastics) were among the most found abundant genera belonging to the phylum of the Proteobacteria. Nonetheless, the composition of proteobacterial communities differed strongly between the west and the east coasts (Fig. 4a, Supplemental Table 5c). Considering plastic, seawater, and sand samples, on east coast *Vibrio* (33.1 %), *Pseudoalteromonas* (25.6 %) and *Photobacterium* (19.3 %) were dominant whereas the genus *Psychrobacter* (39.7 %) was the most abundant on the West coast. Plastic





**Fig. 5.** Total culturable bacterial flora in CFU/g or ml of substrate. Abbreviations: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. Plots represent means, and error bars represent standard deviation of means ( $n = 3$ ) of three independent samplings. The different letters above plots indicate significant differences as determined by Tukey HSD test ( $p \leq 0.05$ ).

samples showed genera compositions different from seawater or sand samples (Fig. 4a, Supplemental Table 5c) with a dominance of *Psychrobacter* (28.8 %), *Photobacterium* (19.5 %), *Pseudoalteromonas* (18.1 %) and *Vibrio* (17.1 %) on plastics, and *Vibrio* (30.1 %), *Pseudoalteromonas* (15.8 %), *Candidatus Pelagibacter* (12.7 %), *Alcanivorax* (12.6 %) and *Alteromonas* (10 %) in seawater and sand. Proteobacterial communities associated with microplastics or found in the water column or on the beach sand were further differentiated using NMDS ordinations and ANOSIM tests (Fig. 4b). The distribution of proteobacterial communities differed significantly (Supplemental Table 5d) between sites (green symbols vs blue symbols on Fig. 4b), material collected (S + SW filled symbols vs PS + PSW empty symbols on Fig. 4b), and the type of sample (combination of both site and material, represented with four ellipses on Fig. 4b). However, the proteobacterial communities found on sand beaches (PS + S) vs coastal waters (PSW + SW) were similar (Fig. 4b; Supplemental Table 5d).

### 3.3. Cultivable bacterial flora analysis and antimicrobial resistance

A dense cultivable bacterial flora was found on the microplastics from Reunion Island ( $3.13 \times 10^7$  colony forming units (CFU)/g of microplastics on average, Fig. 5, Supplemental Table 6). The total cultivable flora was significantly denser on microplastics than in seawater ( $4.82 \times 10^2$  CFU/ml of water) or on sand ( $3.89 \times 10^4$  CFU/g of

sand) whatever the studied site. On the contrary, no differences were found between the density of this culturable flora on microplastics collected on the east or the west coast, or between the seawater and the sand. The fraction of potentially pathogenic bacterial microflora was isolated on selective media: a total of 105 bacterial strains could be identified at the genus level using MALDI-TOF analysis: summarized data are presented in Supplemental Table 7 while numeration data of these strains per genus are presented in Supplemental Table 8. Various genera belonging to the phylum Proteobacteria, Firmicutes and Bacillota were found on plastics, seawater, or sand. On plastics these culturable bacteria reach significantly higher abundances ( $10^3$  to  $10^5$  CFU/g of plastic) than in seawater (1 CFU/ml of seawater) and on sand ( $10^2$  CFU/g of sand). Noteworthy, on plastics from both sites (East or West) and both matrices (seawater or sand), the most dominant bacterial genera were *Exiguobacterium* and *Pseudomonas* with several culturable bacteria scaling from  $10^3$  CFU/g for *Pseudomonas* to  $10^5$  CFU/g for *Exiguobacterium*. Acquired resistance profiles could be sorted for 16 of the 105 strains identified by MALDI-TOF (Table 3 and Supplemental Tables 9a, b). Microplastics carried bacterial strains with AMR, including strains with non-intrinsic resistances to antibiotics. The main antibiotic resistances detected concerned  $\beta$ -lactams such as penicillin, ampicillin and ticarcillin. On plastics, the AMR potential pathogens detected were strains belonging to *Bacillus*, *Enterococcus*, *Pseudomonas* and *Pantoea* genera.

## 4. Discussion

This study contributes to the knowledge of the health risk associated with the plastisphere in an oceanic region still very little documented.

### 4.1. Reunion Island suffers from plastic pollution

Our observation shows that surface coastal waters around Reunion Island are polluted by plastic. Concentrations of  $10,693 \pm 1275$  items/km<sup>2</sup> and  $4025 \pm 4760$  items/km<sup>2</sup> were measured on the West and East coasts respectively. Plastics pollution at Reunion Island may come from local plastic waste sources (Benard and Malet-Damour, 2022) but also from plastic marine debris from the Indian ocean subtropical plastic gyre (Pattiaratchi et al., 2022), with these plastic debris mainly coming from Southeast Asia and carried by the south equatorial current (Thibault et al., 2023). These results are consistent with observations in most other seas and oceans (Thushari and Senevirathna, 2020). In addition, despite of the geographical isolation of Reunion Island in the Indian ocean, the data of the present study argue in favor the hypothesis that (i) marine plastic pollution is a global connected threat to all the world's oceans (Lincoln et al., 2022), and (ii) most scattered oceanic islands are

**Table 3**

Antibiotic multiresistances detected among the culturable bacterial strains. In bold are noticed non-natural antibiotic resistances. Abbreviations: PSW: plastic from sea water; PS: plastic from sand beach; SW: sea water; S: sand beach; TS: trimethoprim/sulfamethoxazole; PT: piperacilin/tazobactam; AC: amoxicillin/clavulanic acid.

Site	Substrate	Bacterial strain MT code	Bacterial genus	ATB1	ATB2	ATB3	ATB4
East	PSW	T2-14	<i>Pantoea</i>	Ampicillin	Cefadroxil	<b>Ticarcillin</b>	
		T3-42	<i>Pseudomonas</i>	TS			
	SW	T5-26	<i>Staphylococcus</i>	<b>Erythromycin</b>	<b>Penicillin</b>		
		T4-54	<i>Bacillus</i>	Amoxicillin	<b>Imipenem</b>	Penicillin G	<b>Vancomycin</b>
		T4-52	<i>Vibrio</i>	<b>Ampicillin</b>			
	PS	A1-2	<i>Pseudomonas</i>	<b>Ticarcillin</b>	TS		
		A2-15	<i>Bacillus</i>	Amoxicillin	<b>Imipenem</b>	<b>Penicillin G</b>	
		A1-13	<i>Vibrio</i>	<b>Ampicillin</b>			
	S	A4-9	<i>Bacillus</i>	Amoxicillin	<b>Penicillin G</b>		
		A6-6	<i>Enterobacter</i>	Ampicillin	AC	Cefalexin	
			<i>Pseudomonas</i>	TS			
	PSW	MEEP141	<i>Pseudomonas</i>	TS			
	SW	E5-28	<i>Pseudomonas</i>	TS			
		E4-13	<i>Bacillus</i>	Amoxicillin	<b>Clindamycin</b>		
West	PS	B1-3	<i>Enterococcus</i>	<b>Rifampicin</b>	Vancomycin		
	S	B6-14	<i>Aeromonas</i>	<b>Piperacilin</b>	<b>Ticarcillin</b>	<b>Ticarcillin/Clavulanic acid</b>	<b>PT</b>
		B5-17	<i>Staphylococcus</i>	<b>Clindamycin</b>	<b>Fusidic acid</b>	<b>Penicillin</b>	

threatened by such a pollution (Derraik, 2002).

However, the concentrations of these stranded plastics on the beaches of Reunion Island (0.35 items/m<sup>2</sup> on the west coast and 0.0223 items/m<sup>2</sup> on the east coast) are lower than reported on other Indian Ocean islands such as the Chagos Archipelago (6 items/m<sup>2</sup>, Hoare et al., 2022) or the Maldives (35.8 items/m<sup>2</sup>, Imhof et al., 2017). Plastic abundance varies with environmental settings such as wind speed, swell intensity, marine currents velocity, seasonality, and the morphology of the island (Imhof et al., 2017). Reunion Island is a young volcanic island (Lenat et al., 2001) harboring few coral reefs and subject to oceanic swell and strong marine currents impacting the few beaches located on the east coast (Pous et al., 2014). Alternatively, the low plastic abundance may result from distinct oceanic influences as well as the remoteness of Reunion Island. The island is mostly under the influence of south Easterly trade winds and currents, and waters circulate from Western Australia to Reunion Island without colliding any important land mass (Schott et al., 2009). Concerning the nature of the plastic polymers found in Reunion Island, whether on the sea surface or in the sand of the beaches, polyethylene and polypropylene are the most abundant polymers. The dominance of these two polymers is hegemonic across the world's seas and oceans as reported in the meta-analysis by Erni-Cassola et al. (2019).

#### 4.2. Marine microplastic debris reaching Reunion Island host a specific microbiome

In the present study we found that a substantial proportion of up to 60 % of the operational taxonomic units (OTUs) was shared between microplastics and their environment (seawater or sand beach). Seawater microplastic debris are colonized by planktonic microorganisms forming a biofilm whose composition presents expected high similarities with seawater microbiome (Zettler et al., 2013; De Tender et al., 2017). Our observation highlights the colonizing role of bacterioplankton but also suggests a relatively new colonization (40 % of OTUs are specific to plastics). Such renewal of microbiome composition is known as resulting from the oceanic environmental factors as temperature, salinity, light, and UV radiations: these factors affect the microbial biofilm composition and its renewal with an enrichment in pathogens (Sooriyakumar et al., 2022; Shan et al., 2022).

Similarly, plastic debris reaching beaches, after being introduced into the marine environment, undergo physical and biological processes that break them down into smaller fragments, which can eventually be incorporated as part of the beach sand (Napper and Thompson, 2020). Through this process, the plastic fragments and the sandy beach substrate can contribute to each other's bacterial enrichment, which the high proportion of shared OTUs may reflect. A recent study has shown that a high proportion of OTUs shared between plastic debris and sand samples suggests that plastic debris have a significant impact on microbial communities in marine sediment (Seeley et al., 2020). Nevertheless, there is still an important proportion of OTUs (up to 38 %) that are specific to the plastic debris indicating that plastics are also a microbial ecological niche for specific communities compared to the communities from the environment. A recent review suggests that such specific communities result from biofilm formation and evolution processes, with an enrichment in bacteria able to use the plastic polymer as carbon source (Sooriyakumar et al., 2022; Zhurina et al., 2022).

#### 4.3. Proteobacteria and Bacteroidota phyla dominate marine microplastic microbiome

The microbial community structure on Reunion Island's plastic pollution exhibits a notable dominance of Proteobacteria and Bacteroidota phyla across various sample types, including seawater-plastic, sand-plastic, seawater, and beach sand. These phyla, particularly Proteobacteria (75 % average relative abundance) and Bacteroidota (11 %), remain consistent irrespective of the substrate or site studied, reflecting

their adaptability to diverse environmental conditions. Proteobacteria and Bacteroidota are diverse groups of bacteria known for their ability to adapt to a wide range of environmental conditions (Kerstens et al., 2006; Hahnke et al., 2016). They are commonly found in the oceans and their high abundance is well documented in seawater and marine sediments (Stal and Cretoiu, 2022). Our observation is consistent with other reports that also observed dominance of Proteobacteria and Bacteroidota phyla associated to plastic biofilm, e.g. Northern Europe seas, Mediterranean Sea and the Atlantic Ocean (Oberbeckmann et al., 2014; Vaksmaa et al., 2021; Debroas et al., 2017).

Within the dominant Proteobacteria phylum, specific dominant genera stand out. On seawater plastics, *Photobacterium* (33 %), *Pseudoalteromonas* (27 %), and *Psychrobacter* (18 %) are highly abundant, comprising up to 78 % of the Proteobacteria OTUs found on plastics. In surrounding seawater, dominant *Proteobacteria* genera, including *Candidatus* (24 %), *Vibrio* (24 %), *Alcanivorax* (18 %), and *Alteromonas* (11 %), collectively represent over 77 % of the OTUs. These significant differences between Proteobacteria found on plastics and in surrounding waters suggest that marine microorganisms have adapted to plastics as a colonization surface (Roager and Sonnenschein, 2019). Moreover, several of these dominant Proteobacteria genera (*Photobacterium*, *Pseudoalteromonas*, and *Psychrobacter*) are known for their ability to biodegrade and utilize plastics as a carbon source and nutrient (Raghul et al., 2014; Muriel-Millán et al., 2021; Atanasova et al., 2021). This presence on seawater plastics indicates that the plastics reaching Reunion Island have likely undergone degradation during prolonged floating, in line with the concept of plastic debris transporting microorganisms as suggested by Pattiaratchi et al. (2022).

The diversity and abundance of Proteobacterial OTUs on plastics and sand from the East coast resemble each other, with dominant genera such as *Vibrio*, *Pseudoalteromonas*, and *Alteromonas*, suggesting a role for plastics in transporting and enriching the sand bacterial community. In contrast, on the West coast, the Proteobacteria community structure significantly differs between sand plastics, dominated by *Psychrobacter* (>80 %), and sandy beach sand, where the Proteobacteria community is led by the genus *Dyella* (>70 %). This variation may be linked to the specific types of polymers present on the plastics reaching each coast, with *Psychrobacter* known for its biodegradation of polyurethane and polycaprolactone polyester (PCL) (Atanasova et al., 2021). The dominance of *Dyella* in beach sand may indicate residual wastewater contamination from forest or cultivated soils, where *Dyella* is primarily isolated (Dar et al., 2020; Huang et al., 2021).

#### 4.4. Cultivable bacteria isolated from marine microplastic debris

The study of cultivable bacteria isolated from marine microplastic debris reveals significant microbial activity, with an average concentration of  $3 \times 10^7$  colony forming units (CFU)/g of plastic. Notably, this demonstrates that microplastic serve as an ecological niche where bacteria remain metabolically active and cultivable. Comparatively, recent research found that plastic microbeads could harbor up to  $60 \times 10^{10}$  CFU/cm<sup>2</sup> of plastic (Türetken et al., 2020). In the present study at Reunion Island, the number of cultivable bacteria on plastics exceeded those in beach sediments by 800 times and coastal waters by up to  $65 \times 10^3$  times.

Similarly, potential pathogens cultivable bacteria were significantly more abundant on plastic debris (ranging from  $10^3$  to  $10^5$  CFU/g of plastic) than in surrounding environments like seawater or sediment (1 to 1000 CFU/g of sand or ml of seawater). This is consistent with Wu et al. (2019) work that showed higher abundance of pathogenic bacterial families on microplastics, compared to substrates. The most encountered potentially pathogens cultivable bacterial strains belong to the *Bacillus*, *Exiguobacterium*, and *Pseudomonas* genera, raising concerns about the potential transmission of infectious pathogens to humans or animals (Bergan, 1981; Mandic-Mulec et al., 2015; Chen et al., 2018). Remarkably, many of these potentially pathogenic bacteria also possess

the capacity to degrade plastics, exemplified by the genus *Pseudomonas*, which is both abundant (up to  $10^5$  CFU/g of plastic) and proficient in plastic degradation (Wilkes and Aristilde, 2017).

#### 4.5. AMRs bacteria

Marine plastics have been shown to enrich the plastisphere with pathogens, including genera like *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Aeromonas*, and the Enterobacterales family, many of which have acquired resistance to antibiotics (Junaid et al., 2022). This poses a significant threat to public health and the environment (Marathe and Bank, 2022). In the Western Indian Ocean, this study represents one of the first reports of isolating cultivable pathogens from plastics for potential antimicrobial susceptibility testing (AST). Resistance to antibiotics was observed in strains of *Bacillus*, *Enterococcus*, *Pseudomonas*, and *Vibrio* isolated from Reunion Island's seawater or sandy beaches. The majority of observed resistance was directed against molecules in the  $\beta$ -lactam family, such as ampicillin, penicillin, and ticarcillin. This is consistent with recent findings that residual beta-lactams, including ampicillin, are common emerging pollutants that chemically adsorb onto plastic, promoting resistance among the plastisphere's bacterial community (Imran et al., 2019; Wang et al., 2021). Furthermore, beta-lactam resistance genes (*bla* genes family) are often associated with mobile genetic elements (MGEs), facilitating horizontal gene transfer within the plastisphere (Wang et al., 2021; Zhang et al., 2022; Silva et al., 2023). This data, collected in a high-income country in the Southwest Indian Ocean, underscores that microplastic debris serves as a reservoir of potentially antibiotic-resistant pathogens (AMR), aligning with similar reports from around the world (Yang et al., 2019; Bowley et al., 2021; Liu et al., 2021). Importantly, this should be viewed from a One Health perspective, acknowledging that highly anthropized coastal environments can be contaminated by enteric bacteria from human or animal sources, potentially including MDR bacteria, based on regional epidemiology (Fernández-Bravo and Figueras, 2020; Miltgen et al., 2022).

## 5. Conclusions

In this study, we presented a first case of the plastisphere assessment from Reunion Island, a remote oceanic island located in the Southwest Indian Ocean, polluted by plastic debris from various geographical origins. The characterization of plastic pollution in the island's coastal waters and beaches indicates that Reunion Island is facing plastic pollution with up to 10,000 objects/km<sup>2</sup> in the coastal waters, mainly consisting of polyethylene (up to 75 %) and polypropylene (up to 25 %). Plastic debris host dense microbiomes, dominated by Proteobacteria (80 %). In addition, the cultivable microbiotes reached  $10^9$  CFU/g of microplastics, with a dominance of bacteria from genera *Exiguobacterium* ( $10^5$  CFU/g of plastic) and *Pseudomonas* ( $10^3$  CFU/g of plastic). This plastic debris also carries  $\beta$ -lactam resistant AMR bacteria such as certain strains of the genera *Bacillus*, *Enterococcus* and *Pantoea* resistant to ampicillin, penicillin and ticarcillin. Overall, our results confirm, as it has already been described for other islands in other oceans and seas, that the islands of the Indian Ocean are facing severe marine plastic pollution, the debris of which host a dense plastisphere including AMR bacteria. Our data also suggests potential risks associated with a plastic-specific microbiome for Southwest Indian Ocean socio-ecosystems.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2023.115911>.

## Data accessibility

NGS raw data 16SrDNA sequences are deposited in zenodo data bank: <https://doi.org/10.5281/zenodo.8063253>.

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## CRediT authorship contribution statement

Conceptualization: PJ; Funding acquisition: PJ, GM, PT, TB; Methodology: PJ, GM, FG, BV; Resources and investigation: SB, MT, LS, GM, PJ, VL; Formal analysis: LS, FG, BV; Data curation and validation: FG, LS, BV, GM; Writing original draft preparation: LS, GM, BV, FG, VL, MT, SB, PT, TB, PJ; Project administration and supervision: PJ. All authors have read and agreed to the published version of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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