Macroplastic-associated antibiotic-resistant bacteria on Portuguese beaches: are there seasonal and polymer-type variations?

Cátia Costa[1, 2, 3], Antunes M. [1], Augier E.[4], Calado M.L.[1], Ferreira G.[1], Oliveira M.A.M.[1], Sale A.[5], Simões M.[1, 6, 7], Henriques I.[2], Tacão M.[3] and Campos M.J.[1]

[1]MARE—Marine and Environmental Sciences Centre/ARNET—Aquatic Research Network Associate Laboratory, School of Tourism and Maritime Technology, Polytechnic Institute of Leiria, 2520-630 Peniche, Portugal;
[2]Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456 Coimbra, Portugal;
[3]CESAM—Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal;
[4]Polytechnic Clermont, Genie Biologique;
[5]UNICA — University of Cagliari, Department of life and environmental science (DISVA);
[6]CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Porto, Portugal;
[7]Department of Biology, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal;

1. Background

The Portuguese coastline is increasingly affected by microbial contamination and plastic pollution, both of which pose risks to environmental and public health[1]. Plastic surfaces can be colonised by several microorganisms, forming a new ecosystem called plastisphere[2] (Fig. 1). Previous studies[3,4,5,6] have already detected antibiotic-resistant bacteria (ARB) and pathogenic bacteria in the plastisphere.

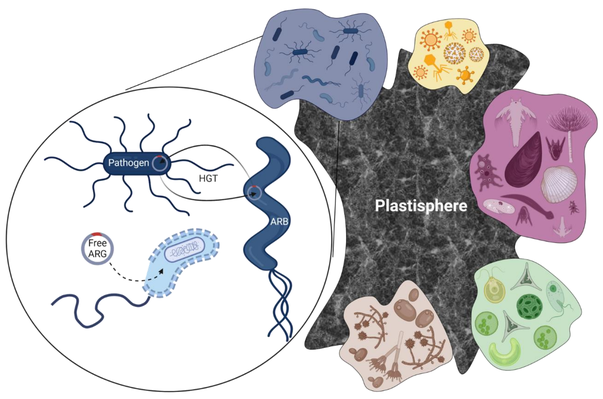


Fig 1. Microbial interactions and Antibiotic-resistant (AR) in the plastisphere contains viruses, bacteria, fungi, microalgae, and invertebrates. The presence and proximity of pathogenic microorganisms increases the potential for horizontal gene transfer. Plastics can act as carriers for AR bacteria and antibiotic resistance genes (ARG). Bacteria can incorporate free mobile genetic elements including plasmids, transposons or integrons, which may contain ARG.

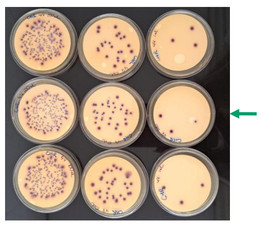
2. Objectives

This study assessed the **presence of ARB on macroplastics** collected from **Portuguese beaches**, focusing **on seasonal variations** and **differences among polymer types**.

3. Methodology

- Total Coliforms (TC)
- Faecal Coliforms (FC)
- Enterococci

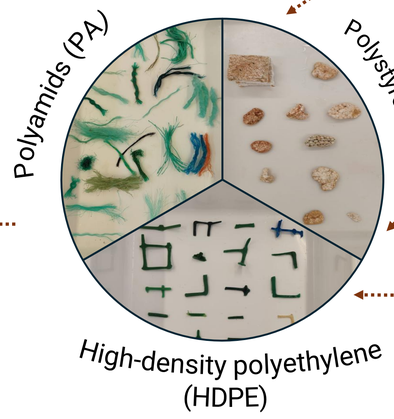
Colony-Forming Unit (CFU).100 mL^{-1}



Aveiro (A1 and A2)

Figueira da Foz (F1 and F2)

Peniche (P1 and P2)



Amoxicicilin, Cefotaxime, Ciplofloxacin, Colistin, Gentamicin, Meropenem

✚

TCBS agar (30°C) → AR *Vibrio*

mFC agar (37°C) → AR Enterobacterales

CFU.g^{-1}

Biofilm suspension

4. Results

Results are shown in Fig. 1 and 2.

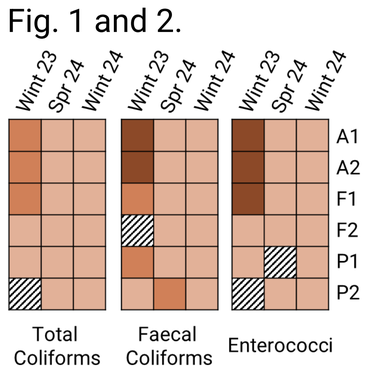


Fig 2. CFU.100 mL^{-1} of seawater during three seasons (Winter 2023, Spring and Winter 2024). The scale represents N.D. (Not Defined), recommended, acceptable and exceeds acceptable maximum[7].

Water Quality

- ✓ **FC and Enterococci levels exceeded legal limits in Winter 2023 in several beaches**
- ✓ **These values decreased more than 200-fold in Spring and Winter 2024.**

AR Bacteria

- ✓ **In PS, Enterobacterales counts were consistently higher than in PA and HDPE.**
- ✓ **The AR Enterobacterales were consistently high in A1 and P1 beaches, during Winter 2023 and Spring 2024.**
- ✓ **AR Enterobacterales were less frequent in colistin and gentamicin.**
- ✓ **AR *Vibrio* were generally not very abundant, with a few exceptions.**
- ✓ **Ciprofloxacin was the antibiotic with the least common AR *Vibrio*.**

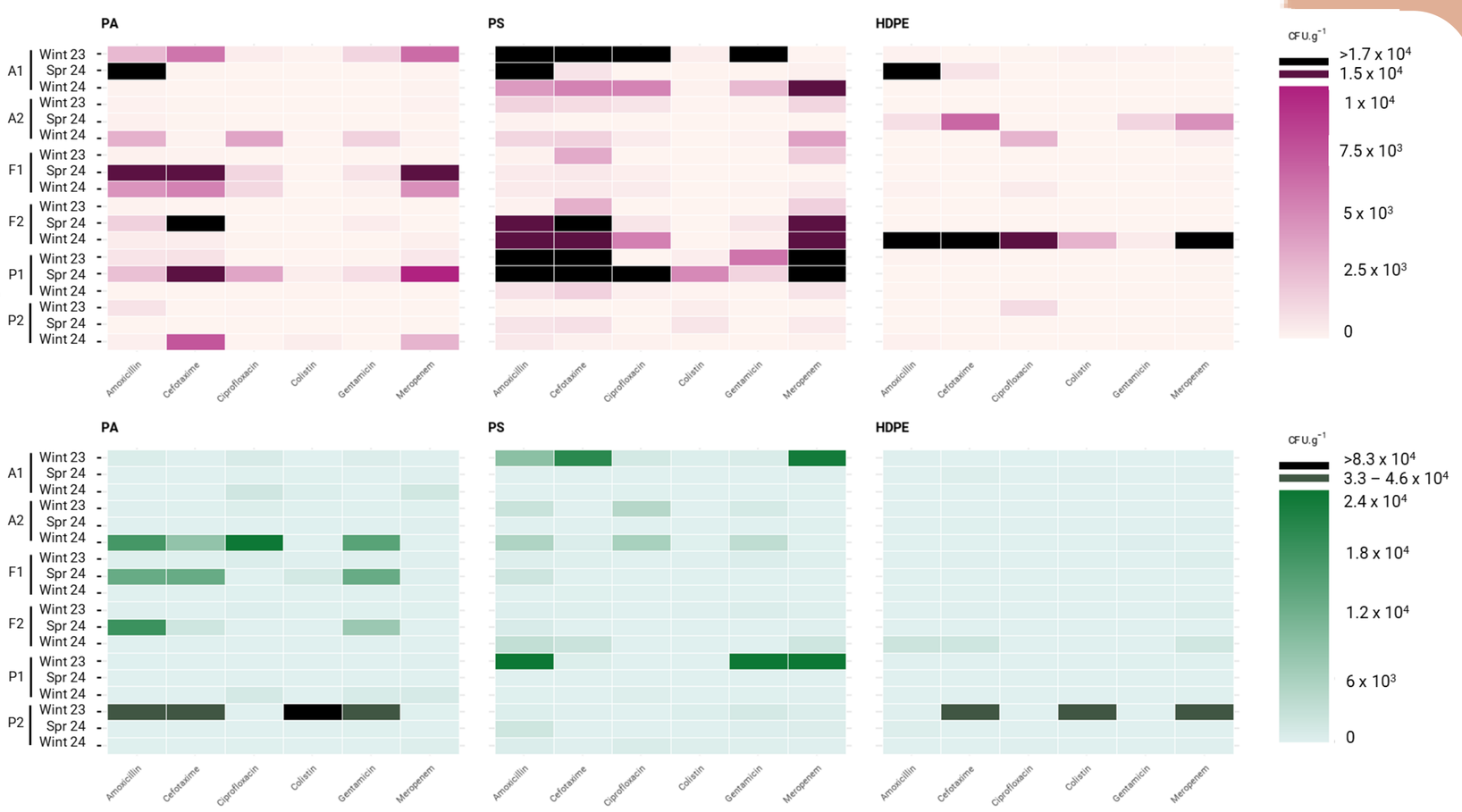


Fig 3. The CFU per g of macroplastic (PA, PS and HDPE) in mFC (in pink) and TCBS (in green) media supplemented with six antibiotics (amoxicillin, cefotaxime, ciprofloxacin, colistin, gentamicin, and meropenem). Data are shown across various sampling sites and seasons. Black cells represent saturation points of >1.7 × 10⁴ CFU·g⁻¹ (mFC) and >8.3 × 10⁴ CFU·g⁻¹ (TCBS).

References

These findings highlight the presence of ARB on marine macroplastics from six beaches on the Portuguese coast. Although no clear seasonal pattern was observed, differences were detected among the types of polymers.