Antibiotic resistant biofilm forming bacteria on plastic samples from Antarctic environment: An emerging reservoir of antibiotic resistance genes in polar environments

Bacterias formadoras de biopelículas resistentes a antibióticos en muestras de plástico procedentes del entorno antártico: un reservorio emergente de genes de resistencia a antibióticos en ambientes polares

Soumya Kavya Bhavan[1], Ammanamveetil Abdulla Mohamed Hatha[1, a]

[1] Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin – 682 016, Keralam, INDIA.

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[a] Corresponding author details:
Dr. Ammanamveetil Abdulla Mohamed Hatha
Emeritus Professor
Dept. of Marine Biology, Microbiology and Biochemistry
School of Marine Sciences
Cochin University of Science and Technology,
Cochin – 682016 Keralam, India.

Abstract

Antarctica remains one of the most isolated and pristine regions on the planet; nevertheless, plastic contamination has recently been reported in its ecosystems and associated organisms. There are several reports stating that plastics colonized by viruses or bacteria may be potentially pathogenic. Accordingly, we analyzed the polyethylene and polypropylene macroplastic samples collected from the Antarctic region for the bacterial abundance by the standard plate count method. Scanning electron microscopy (SEM) images revealed the presence of bacteria on macroplastic samples. A total of 53 bacterial strains were isolated which were tested for their ability to form biofilms. These biofilm forming bacteria were tested for prevalence of antibiotic resistance which revealed high prevalence of multiple antibiotic resistance among bacterial isolates from Antarctic macroplastic debris. Resistance to ampicillin, amoxicillin, chloramphenicol, ceftazidime, cephalothin, co-trimoxazole, and trimethoprim was common, with approximately 48.6% of isolates identified as multidrug resistant (MDR), with 57.14% carrying *bla*$_{TEM}$, 40% harboring *bla*$_{SHV}$, and 34.28% positive for the *hlyA* gene. The present study highlights the environmental impact of anthropogenic plastic litter, even in the pristine, Antarctic continent. Besides posing risks to flora and fauna, plastics may act as ideal substrates for colonization and prolonged dissemination of biofilm forming, multidrug-resistant bacteria. This illustrates the urgent need for global monitoring and intervention strategies to address the spread of antimicrobial resistance in polar environments.

Keywords: plastics, biofilms, antibiotic resistance, Antarctic environment

Resumen

La Antártida sigue siendo una de las regiones más aisladas y prístinas del planeta; sin embargo, recientemente se ha reportado contaminación por plásticos en sus ecosistemas y en los organismos asociados a ellos. Existen diversos informes que indican que los plásticos colonizados por virus o bacterias pueden ser potencialmente patógenos. En consecuencia, analizamos muestras de macroplásticos de polietileno y polipropileno recolectadas en la región antártica para determinar la abundancia bacteriana mediante el método estándar de recuento en placa. Las imágenes de microscopía electrónica de barrido (MEB) revelaron la presencia de bacterias en las muestras de macroplásticos. Se aislaron un total de 53 cepas bacterianas, las cuales fueron evaluadas por su capacidad para formar biopelículas. Estas bacterias formadoras de biopelículas se analizaron para determinar la prevalencia de resistencia a antibióticos, revelando una alta prevalencia de multirresistencia entre los aislados bacterianos provenientes de residuos de macroplásticos antárticos. Se observó una resistencia común a ampicilina, amoxicilina, cloranfenicol, ceftazidima, cefalotina, cotrimoxazol y trimetoprima; aproximadamente el 48,6 % de los aislados se identificaron como multirresistentes (MDR), con un 57,14 % portador del gen *bla*$_{TEM}$, un 40 % con el gen *bla*$_{SHV}$ y un 34,28 % positivo para el gen *hly*A. El presente estudio pone de relieve el impacto ambiental de los residuos plásticos de origen antropogénico, incluso en el continente antártico, un entorno prístino. Además de representar riesgos para la flora y la fauna, los plásticos pueden actuar como sustratos ideales para la colonización y la propagación prolongada de bacterias multirresistentes formadoras de biopelículas. Esto ilustra la urgente necesidad de implementar estrategias globales de monitoreo e intervención para abordar la propagación de la resistencia a los antimicrobianos en los entornos polares.

Palabras Clave: plásticos; biopelículas; resistencia a antibióticos; medioambiente antártico

Introduction

The extensive production and improper management of plastics have resulted in widespread plastic pollution across global ecosystems. Each year, approximately 360 million tons of plastic are produced, yet only about 7% is recycled, leaving the majority to accumulate in the environment (Thacharodi *et al*, 2024). Despite its remoteness and low levels of human activity, the Antarctic continent and the Southern Ocean are not immune to plastic pollution. Most plastics found in these areas include packaging bands, synthetic threads, and fishing nets (Zhang *et al*, 2020). There is substantial evidence that plastics pose significant threats to marine ecosystems, causing direct health effects such as entanglement and ingestion by marine species, often resulting in injury, impaired mobility, and increased mortality.

One of the defining characteristics of plastics in the marine environment is their ability to act as substrates for the attachment and transport of organisms, a phenomenon that may facilitate biological dispersal and even species invasions across oceanic regions (Amaral-Zettler *et al*, 2020). Such processes have been documented in remote locations, including the Antarctic Peninsula, where rafting organisms associated with plastic debris have been reported (Barnes, 2002). Once introduced into seawater, plastics are rapidly colonized by microorganisms (Oberbeckmann *et al*, 2014), giving rise to biofilm assemblages collectively referred to as the "Plastisphere" (Zettler *et al*, 2013). Importantly, these assemblages are taxonomically diverse, encompassing a wide range of prokaryotic taxa such as bacteria and archaea (Debroas *et al*, 2017; Bryant *et al*, 2016; Dussud *et al*, 2018). Micro and macroorganisms colonize plastic surfaces through a classical ecological succession, beginning with rapid biofilm formation and progressing to complex biofouling communities over time (Bryant *et al*, 2016; Jacquin et al, 2019; Rogers *et al*, 2020). In addition to shaping microbial diversity, biofouling alters the physicochemical properties of plastics, influencing their rates of weathering and their capacity to adsorb environmental contaminants (Rummel *et al*, 2017). Biofilm growth can also modify the buoyancy of plastic debris, promoting vertical transport through the water column and ultimately affecting its environmental fate (Kaiser *et al*, 2017). The colonization of microplastics is influenced by both abiotic and biotic factors, with key drivers of plastisphere community composition including geographic location (Amaral-Zettler *et al*, 2020; Caruso *et al*, 2024; Oberbeckmann *et al*, 2014), seasonality, and temporal variation (Oberbeckmann *et al*, 2014; Jacquin *et al*, 2019).

Recent research reveals that plastisphere microbial communities in Antarctic waters harbor not only diverse environmental bacteria but also antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Caruso *et al*, 2024; Zhai *et al*, 2023). The adsorption of antibiotics onto plastics, combined with biofilm protection, creates environmental niches facilitating antimicrobial resistance proliferation in these fragile ecosystems (Stevenson *et al*, 2024; Scott *et al*, 2020). Multiple studies report widespread multidrug resistance among bacteria isolated from the plastisphere in Antarctic waters, including resistance to cephalosporins, quinolones, and β-lactams, with both natural and anthropogenic factors contributing to the resistome (Caruso *et al*, 2024; Laganà *et al*, 2019). Furthermore, migratory species such as whales, seals, penguins, and albatrosses interact with plastic debris and potentially act as vectors, dispersing human-associated resistant bacteria across vast marine regions (Croxall *et al*, 2005; Murphy *et al*, 2021; Cerdà *et al*, 2018). This highlights the wider ecological and anthropogenic implications of plastisphere biofilms in remote environments where plastics may facilitate the spread of resistant and pathogenic microorganisms. As plastics serve as durable substrates for diverse microbes, they alter the natural balance by creating new microhabitats that promote microbial survival and dispersal, including taxa with virulence and resistance traits observed both in Antarctic waters and associated fauna (Lacerda *et al*, 2025; Zhai *et al*, 2023).

Accordingly, this study was carried out to expand knowledge of the microbial community composition associated with plastic debris collected from the Antarctic region. The ability of the plastic associated bacteria to form biofilms and the prevalence of antibiotic resistance among the isolates was also studied.

Materials and methods

Description of study area and Sample collection

The sampling area was situated near the Indian Antarctic research station Maitri, (70°45'52" S, 11°44'03" E) located within the Schirmacher Oasis, East Antarctica. This region is an inland, ice-free rocky plateau characterized by a polar desert climate, persistently sub zero temperatures, and seasonal freshwater melt-water lakes (National Centre for Polar and Ocean Research, 2026; Huang *et al*, 2013). Location map of the study region is represented in Figure 1. The sampling was conducted during the January-March 2020 period as part of the 39th Indian Scientific Expedition to Antarctica (39th ISEA). Owing to the remoteness of the region and the limited occurrence of plastic debris all plastics during the field survey were collected. A total of two macroplastic fragments measuring approximately 5.5 cm and 7 cm in length were recovered. Their dimensions were measured using a ruler, and the items were aseptically retrieved from the sediment using sterile forceps to minimize external contamination. Excess loosely attached sediment was gently removed from the surface of each macroplastics. The samples were individually transferred into sterile zip- lock bags for storage and transport. Upon receipt in the laboratory, samples were stored at 4 °C until further processing for microbial analysis and isolation of macroplastic associated bacterial communities.

Characterization of the macroplastics

Chemical characterization of the macroplastic samples was performed using Fourier Transform Infrared Spectroscopy (FT-IR) on a PerkinElmer FT-IR Spectrum Two spectrometer at the Department of Chemical Oceanography, Cochin

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Figure 1. Location of the study region. Indian Antarctic research station, Maitri : The geographical coordinates of the study region - 70°45'52" S, 11°44'03" E)

University of Science and Technology (CUSAT). Samples were analyzed directly without further preparation. Spectra were recorded over the range of 4000–600 cm^{-1} with a spectral resolution of 4 cm^{-1}, and each spectrum was averaged over 50 scans. Background spectra were collected before each measurement and automatically subtracted. The obtained spectra were compared to standard reference spectra to identify polymer types based on their characteristic functional group peaks (Vimala and Mathew, 2016; Jung *et al*, 2018).

The surface morphology of macroplastic samples were examined using Scanning Electron Microscopy (SEM). Imaging was performed with a JSM-6390 Scanning Electron Microscope at Sophisticated Testing and Instrumentation Centre (STIC), CUSAT, India. Samples were observed at various magnifications to evaluate surface topography and identify signs of weathering or microbial colonization through biofilm development.

Isolation of plastic bound bacteria

Macroplastics collected from Antarctica were thoroughly washed with sterile water to remove any attached debris. The plastic samples were aseptically cut into small pieces and suspended in 10 mL of sterile isotonic saline solution (0.85% NaCl) and shaken vigorously to dislodge bacteria from plastic pieces. Six serial ten-fold dilutions (10^{-1} to 10^{-6}) were subsequently prepared. One hundred microliters of the sample were plated onto sterile Zobell marine agar plates, the plates were incubated for 24-72 hours at 20 °C before colony enumeration. The colonies that developed on the Zobell marine agar plates were counted, and total plate count was expressed as the number of colony-forming units (CFU/mL). Thereafter, distinct bacterial colonies were isolated based on their morphology and stored in Zobell marine agar slants for further examination. Gram staining and biochemical tests were performed according to standard microbiological procedures for preliminary identification of the isolates.

Preliminary characterization of the isolates

Preliminary characterization of the isolates was performed based on the colony morphology, Gram staining, motility and biochemical tests. The biochemical characterization included oxidase, catalase, and the IMViC tests (indole production, Methyl red, Voges-Proskauer, and citrate utilization) following the standard microbiological procedures described in Bergey's Manual of Determinative Bacteriology (Holt *et al*, 1994).

Molecular characterization of the bacteria

Extraction of genomic DNA

Genomic DNA was extracted from pure bacterial cultures using the DNeasy extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The quality and integrity of the extracted DNA were assessed prior to downstream molecular analysis.

16S rRNA gene amplification by PCR

Molecular characterization of each isolate was performed using 16S rRNA analysis with extracted genomic DNA as a template. The universal primer pair (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′) was used to amplify the bacterial 16S rRNA via polymerase chain reaction (PCR) (Bosshard *et al*. 2000). PCR amplification was carried out under the following conditions: an initial denaturation at 95 °C for two minutes, followed by 30 cycles of denaturation (95 °C for one minute), annealing (58 °C for one minute), and extension (72 °C for two minutes), with a final extension step at 72 °C for 10 minutes. PCR products were identified using agarose gel electrophoresis in Tris (tris(hydroxymethyl)aminomethane), borate, and EDTA (ethylenediaminetetraacetic acid) (TBE) buffer containing 0.5 µg/mL ethidium bromide.

Sequencing and Phylogenetic analysis

The PCR products were sequenced using an ABI 3730 XL Genetic Analyzer (Applied Biosystems, USA). The obtained 16S rRNA gene sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) (National Centre for Biotechnology Information, 2026) to identify the closest taxa. Phylogenetic trees were constructed using two tree-building algorithms: maximum likelihood (ML) and neighbor-joining (NJ) approaches in MEGA version 11 (Tamura *et al*, 2013). The 16S rRNA gene sequences were aligned using CLUSTAL W.

Evaluation of biofilm forming ability of isolated bacteria

Biofilm forming capacity of bacteria was checked. The bacterial isolates were inoculated into Luria–Bertani (LB) broth and cultured for 24 - 48 hours at 20 °C. An inoculum containing approximately 10^6 CFU/mL was prepared to

check biofilm formation. Using a micropipette, 200 µL of the bacterial suspension was dispensed into each well of a sterile, flat-bottom 96-well microtiter plate. Wells containing only sterile medium (no bacteria) were maintained as a negative control. Three or more replicates were prepared for each experimental condition and incubated at 20 ºC for 24 hours. Two hundred microliters of sterile LB were used as a negative control in each microtiter plate. After incubation, each well was washed three times with sterile isotonic saline solution (0.85% NaCl) to remove loosely adhered cells. The cells adhered to the wells were fixed with 200 µL of 99% methanol (15 Minutes) and the microplate was air dried. The cells were stained with 200 µl of crystal violet solution (0.1%) for 5 minutes. The dye was drained and rinsed with tap water, followed by drying of the microplate. Finally, the remaining dye in the well was resolubilized in 200 µL of 30% glacial acetic acid. The negative control was processed in the same way as the test samples. The optical density (OD) was measured using a microplate reader (Varioskan® LUX Microplate Reader, Thermo Scientific, Waltham, USA) at a wavelength of 550 nm. The ODcut was calculated as the Mean OD of Negative Control plus three times its Standard Deviation (SD) (ODcut=Mean OD of Negative Control + 3 ×SD of OD values of Negative Control). Based on this, biofilm production is categorized as follows: No biofilm production: OD ≤ ODcut, Weak biofilm production: ODcut < OD≤ 2 × ODcut, Moderate biofilm production : 2 × ODcut < OD≤ 4 × ODcut, and Strong biofilm production: ODt > 4 × ODcut (Stepanovic *et al*, 2000; Singh *et al*, 2017).

Antibiotic susceptibility testing

Selected bacterial isolates were tested for their antibiotic susceptibility using the agar disc diffusion method (Bauer *et al*, 1966) on Mueller Hinton agar (Hi-Media, Maharashtra, India). The following antibiotics were used for susceptibility testing: ampicillin (Amp,10 mcg), amoxicillin (Amx, 30 mcg), Carbenicillin (Cb, 100 mcg) Ceftazidime (Caz, 30 mcg), Cephalothin (Cep, 30 mcg), Cefpodoxime (Cpd, 10 mcg), Co Trimoxazole (Cot, 25 mcg), Ciprofloxacin (Cip, 5 mcg), Chloramphenicol (C, 30 mcg), Colistin (Cl, 30 mcg) Gentamicin (Gen, 30 mcg), Streptomycin (S, 10 mcg), Tetracycline (Te, 30 mcg) and Trimethoprim (Tr, 5 mcg). Pure bacterial isolates were grown overnight in 10 ml of nutrient broth at 20 ºC. For each isolate, the inoculum was adjusted to a 0.5 MCFarland standard (approximately 1.5×10^8 CFU/mL). The standardized bacterial suspension was uniformly inoculated onto the surface of sterile, dried Mueller Hinton agar plates using a sterile cotton swab. After five minutes of drying, antibiotic discs were aseptically placed on the seeded Mueller Hinton agar plates, ensuring sufficient separation to prevent overlapping of inhibition zones. The plates were incubated at 20 ºC for 24h. After incubation, the diameters of the inhibition zone were measured. The inhibition zone diameters were interpreted using the Hi-Media interpretative chart based on CLSI (Clinical and Laboratory Standards Institute) and EUCAST (The European Committee on Antimicrobial Susceptibility Testing) guidelines as a standardized reference for comparative phenotypic assessment of antimicrobial susceptibility. The isolates were categorized as sensitive (S) intermediate (I) or resistant (R) (Vishnupriya *et al*. 2024).

Multiple Antibiotic Resistance (MAR) indexing of the isolates

The MAR index was calculated for each bacterial isolate to assess the extent of antibiotic resistance. The MAR index was calculated using the formula: MAR index = Number of antibiotic to which the test isolate resistant/ /Total number of antibiotics used. A MAR index ≥ 0.2 was considered indicative of isolates originating from high-risk sources with frequent antibiotic exposure. This analysis helped identify multidrug resistant isolates and evaluate potential health risks (Krumperman, 1983).

Multidrug resistance Analysis

Multidrug resistance (MDR) was defined according to the internationally accepted criteria proposed by Magiorakos *et al*. (2012), i.e., acquired non susceptibility to at least one agent in three or more antimicrobial categories. The resistance profiles of all isolates were recorded and analyzed to assess the prevalence of MDR among different biofilm forming groups.

Detection of bla$_{SHV}$, bla$_{TEM}$ antibiotic resistance genes and hlyA virulence gene

Selected bacterial isolates were screened for the presence of antibiotic resistance genes such as, *bla*$_{SHV}$, and *bla*$_{TEM}$ and virulence gene *hlyA* by PCR. The primers sequences were as follows; *bla*$_{SHV}$ (F: 5′-AGCCGCTTGAGCAAATTAAAC-3′, R: 5′-ATCCCGCAGATAAATCACCAC-3′; 713bp), *bla*$_{TEM}$ (F:5′-CATTTCCGTGTCGCCCTTATTC-3′, R:5′-CGTTCATCCATAGTTGCCTGAC-3′; 800bp) and *hlyA* (F:5′-GCATCATCAAGCGTACGTTCC-3′, R:5′-AATGAGCCAAGCTGGTTAAGCT-3′; 534bp) as described by Dallenne *et al*. (2010) and Schmidt *et al*. (1995). The optimized protocol was carried out with a PCR mix of 20µL final volume were prepared with 1 µL of total DNA, 1 µM of each primer, and 10 µL of EmeraldAmp® GT PCR Master Mix (Takara, Tokyo, Japan). Amplified PCR products were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide (GeNei, Bangalore, India), and visualized by Gel Documentation System (BioRad Gel DocTM EZ Imager, Hercules, USA).

Statistical analysis

Antibiotic resistance profiles, converted into quantitative data, were averaged per each category of assayed antibiotics and then elaborated by correlating them with the MAR index values using the Microsoft Excel software program (version 10). The association between biofilm forming category (weak, moderate, and strong) and MDR status was assessed using the chi-square test. The relationship between biofilm forming category (weak=1, moderate= 2 and strong=3) and the MAR index was evaluated using Spearman's rank correlation coefficient. A two- tailed p- value <0.05 was considered statistically significant. These analyses were conducted using JASP software (version 0.96.0.0).

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Results

FT-IR profile and Scanning Electron Microscopy (SEM) images of the plastic

The two macroplastic samples were analyzed using FT- IR. The obtained spectra matched the standard spectra of polyethylene (PE) and polypropylene (PP) as shown Figure 2. The FT-IR spectrum of PE exhibited prominent C–H stretching vibrations occurred near 2916 cm^{-1} and 2848 cm^{-1}, indicative of methylene ($–CH_2–$) groups present along the polymer backbone. Strong bending and deformation bands were observed at approximately 1472 cm^{-1} and 720 cm^{-1}, which are signature peaks for the $–CH_2–$ scissoring and rocking modes in PE. Absence of significant carbonyl (C=O) stretching bands around 1700 cm^{-1} suggests minimal oxidation or additive presence. These spectral features confirm that the sample primarily consists of PE, with no indication of other polymer types.

Prominent C–H stretching vibrations are observed at 2949, 2917, 2868, and 2837 cm^{-1}, corresponding to methyl and methylene groups typical of polypropylene's hydrocarbon backbone. A noticeable absorption band at 1746 cm^{-1} indicates the presence of a carbonyl group, likely due to minor oxidative degradation or additives. Bending and deformation vibrations of CH_2 and CH_3 groups appear distinctly at 1456, 1376, and 1359 cm^{-1}. Additionally, absorption peaks between 1256 and 1102 cm^{-1} are attributed to C–C and C–H bending modes characteristic of PP. Several peaks in the lower wavenumber region (997, 973, 899, 841, and 808 cm^{-1}) reflect the isotactic crystallinity of the polymer. Collectively, these spectral features confirm the polymer's identity as PP with minimal surface oxidation (Jung *et al*, 2018; Durowaye *et al*, 2019).

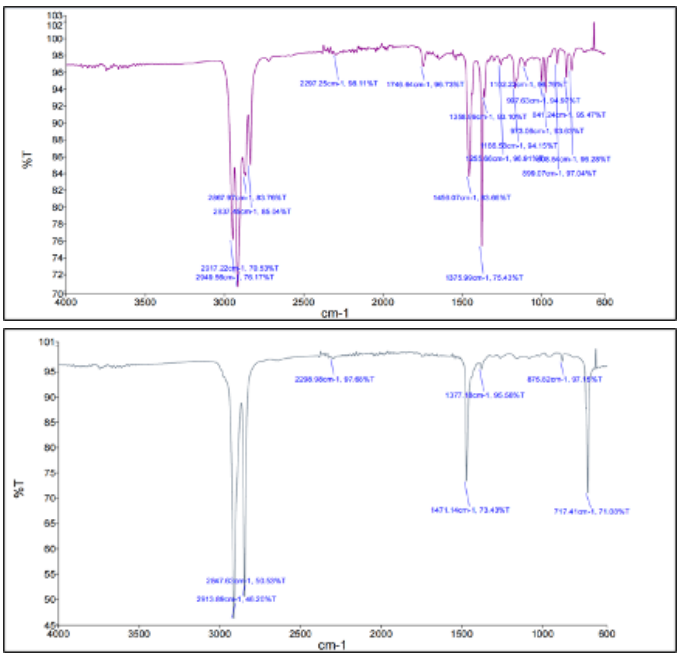


Figure 2. FT-IR spectra of polymer samples illustrating characteristic absorption bands. (X axis - wavenumbers cm-1, Y axis - transmittance) of a) Polypropylene and b) Polyethylene

The SEM analysis of collected macroplastic samples revealed a rough and uneven surface. Microbial cells attached to the plastic surface were also observed, suggesting colonization and biofilm formation on the macroplastic samples collected from Antarctic continent (Figure 3).

Bacterial strains encountered on macroplastics

The bacterial counts associated with Antarctic macroplastic samples were 3.3×10^5 CFU/ mL for PE and 2.4×105 CFU/ mL for PP. A total of 53 morphologically distinct bacterial isolates were recovered, comprising 35 isolates from PE sample and 18 from the PP sample.

Preliminary morphological and biochemical characterization of bacterial isolates

Preliminary characterization using colony morphology, Gram staining and biochemical tests revealed considerable phenotypic diversity among the isolates. Among the 53 isolates 52.8% were gram positive and 47.2% were gram negative. The colonies exhibited a range of colors, including cream, white, light brown, brick red, pink yellow and orange. The bacterial isolates also differed in colony size shape margin, elevation and texture. Biochemical characterization using catalase, oxidase, IMVic, motility, TSI, tests revealed diverse phenotypic characteristics, indicating the presence of diverse bacterial groups. Supplementary Tables S1 and S2 summarize the preliminary characterization of bacterial isolates recovered from PE and PP samples.

Molecular identification and phylogenetic analysis

Two representative bacterial isolates each from the PP and and PE samples were selected for molecular identification by 16S rRNA gene sequencing. High quality 16S rRNA gene sequences were obtained and compared with sequences available in NCBI GenBank database using the BLAST. The representative isolates exhibited >98%

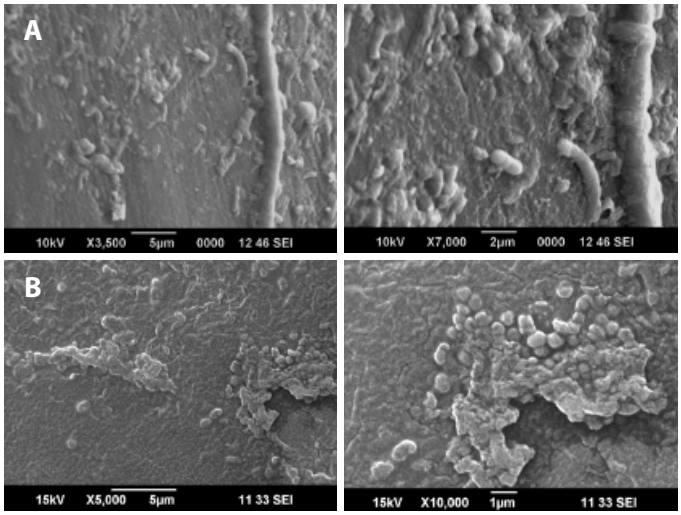


Figure 3. SEM images showing rich microbial community (Plastisphere) present on macroplastic. A) polypropylene B) polyethyene

sequence similarity with *Acinetobacter baumannii* (isolate ID:APE15), *Planococcus maritimus* (isolate ID:APE13), *Priesta aryabhattai* (isolate ID:APP16) and *Bacillus cereus* (isolate ID:APP5),confirming their taxonomic identities. The obtained sequences were deposited in Genbank database under accession numbers PZ457975, PZ4457977, PZ457982, and PZ457992 respectively. The maximum likelihood phylogenetic tree illustrating the taxonomic relationships among the representative isolates and their close reference sequence is shown in Figure 4.

Ability of bacteria to form biofilms on plastic samples

A total of 53 isolates were evaluated for their ability to form biofilms on macroplastics. Most of them were able to form biofilms on plastic (66%). Among those who are capable of forming biofilms, around 21% were strong biofilm producers, while 28% them were moderate biofilm producers and 17 % were weak biofilm producers (Figure 5).

Antibiotic resistance of isolates from plastic samples

A total of 35 (which had the ability to form biofilm) isolates were subjected to sensitivity testing against 14 antibiotics.

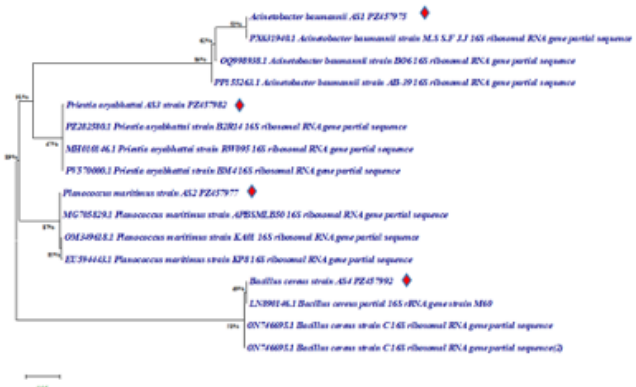


Figure 4. Phylogenetic tree (Mega version 11) of *Acinetobacter baumannii* (PZ457975), *Planococcus maritimus* (PZ4457977), *Priesta aryabhattai* (PZ457982) and *Bacillus cereus* (PZ457992), confirming their taxonomic identities isolated from Antarctic plastic based on the 16S rRNA sequencing.

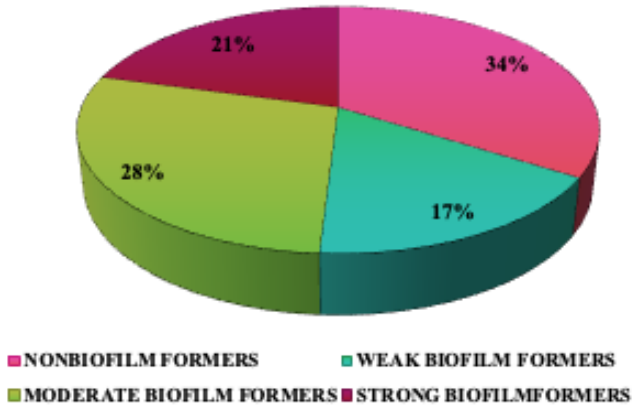


Figure 5. Percentage incidence of biofilm forming bacteria isolated from the plastic samples encountered in Antarctica

The results revealed varying degree of antibiotic resistance among the isolates. Some of them showed resistance to antibiotics like ampicillin, ceftazidime, colistin, streptomycin and cephalothin etc. Figure 6 represents the prevalence of antibiotic resistance (%) among weak, moderate and strong biofilm formers.

Multidrug resistance patterns and Multiple Antibiotic Resistance index among bacterial isolates

Multidrug resistance was observed in approximately 44.4%, 46.6% and 54.5% of weak, moderate, and strong biofilm formers, respectively, with an overall MDR rate of 48.57% (Figure 7). MAR indices of the isolates ranged from 0.07 to 0.78 (Table 1). A MAR index ≥ 0.2 is generally considered indicative of isolates originating from environments with high-risk source of contamination such as human, poultry and animal production systems where antibiotics are often used. High MAR values (>0.2) indicate that these bacteria have likely been exposed to environments with significant antibiotic selection pressure, despite the remote nature of the Antarctic region.

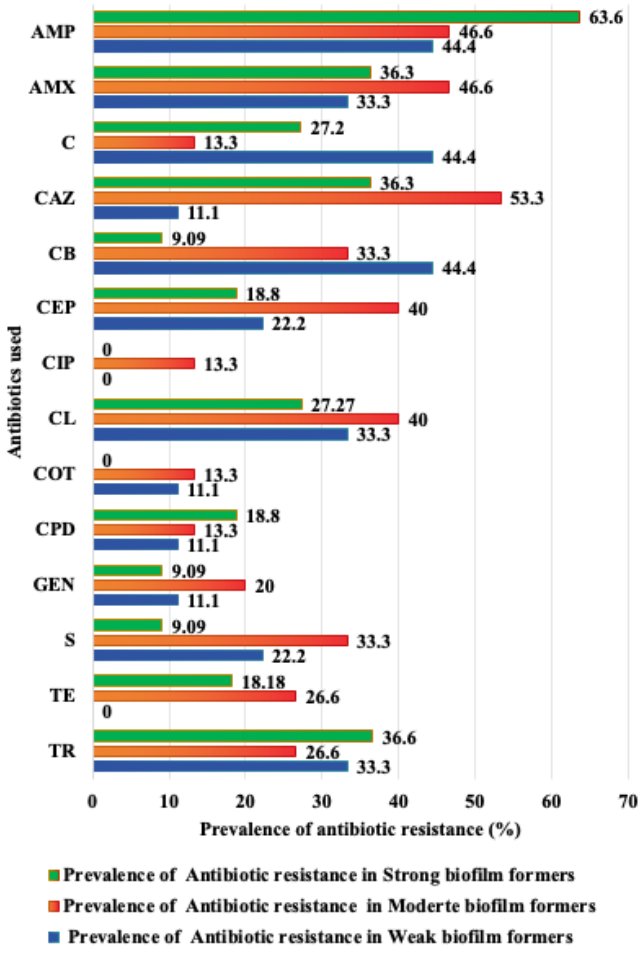


Figure 6. Prevalence of antibiotic resistance (%) among weak, moderate and strong biofilm formers. (AMP = Ampicillin, AMX = amoxicillin, C = Chloramphenicol, CAZ = Ceftazidime, CB = Carbenicillin, CEP = Cephalothin, CPD = Cefpodoxime, COT = Co-Trimoxazole, CIP = Ciprofloxacin, Cl = Colistin, GEN = Gentamicin, S = Streptomycin, TE = Tetracycline, and TR = Trimethoprim)

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Table 1: Multiple antibiotic resistance (MAR) index and resistance patterns of biofilm forming isolates

Isolate ID	Biofilm forming category	MAR Index	Resistance pattern	Risk category
APE14	Weak biofilm formers	0.36	AMP, CB, CL, GEN, TR	High
APE22		0.14	C, AMP	Low
APE27		0.21	CPD, C, AMX	High
APE32		0.14	AMP, CB	Low
APE34		0.42	TR, S, CL, C, AMX, AMP	High
APP1		0.35	TR, COT, C, CEP, AMX	High
APP6		0.07	CB	Low
APP7		0.35	S, CL, CEP, CB, AMP	High
APP13		0.07	CAZ	Low
APE2	Moderate biofim formers	0.14	AMP, CB	Low
APE 6		0.14	C, CAZ	Low
APE9		0.21	S, CAZ, C	High
APE 13		0.07	CAZ	Low
APE16		0.07	AMP	Low
APE17		0.07	AMX	Low
APE31		0.35	AMP, AMX, CAZ, TR, CL	High
APE33		0.35	TE, TR, CL, AMX, AMP	High
APE35		0.28	TE, CEP, AMX, AMP	High
APP2		0.14	TR, CIP	Low
APP4		0	-	Low
APP8		0.78	AMP, AMX, CAZ, CB, CIP, CL, CPD, GEN, S, TE, TR	High
APP9		0.57	TE, S, GEN, CPD, CEP, CB, AMX, CAZ	High
APP11		0.57	S, COT, CL, CEP, CB, CAZ, AMX, AMP	High
APP16		0.5	CAZ, CB, CL, COT, CPD, GEN, S	High
APE1	Strong biofilm formers	0	-	Low
APE3		0.07	AMP	Low
APE8		0	-	Low
APE11		0.28	S, CPD, C, AMP	High
APE15		0.42	TR, CL, CEP, C, AMX, AMP	High
APE20		0.14	CAZ, C	Low
APE21		0.14	AMP, CAZ	Low
APP3		0.28	GEN, CPD, CB C	High
APP5		0.35	TR, CL, CAZ, AMX, AMP	High
APP14		0.35	TR, TE, CL, AMX, AMP	High
APP17		0.35	TR, TE, CEP, AMX, AMP	High

AMP= Ampicillin, AMX= amoxicillin, C= Chloramphenicol, CAZ= Ceftazidime, CB= Carbenicillin, CEP= Cephalothin, CPD= Cefpodoxime, COT= Co- Trimoxazole, CIP= Ciprofloxacin, Cl= Colistin,GEN= Gentamicin, S= Streptomycin, TE= Tetracycline, and TR = Trimethoprim

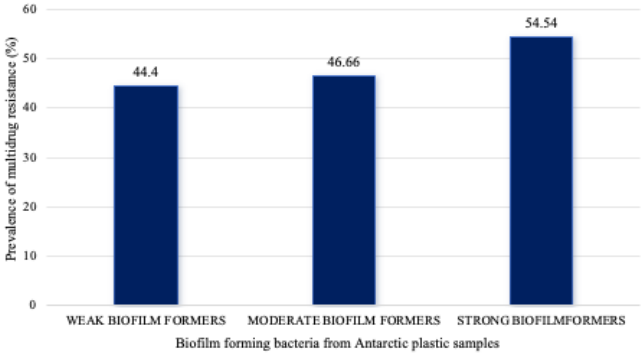


Figure 7. Overall prevalence of multidrug resistance among bacterial isolates with capability to form biofilms.

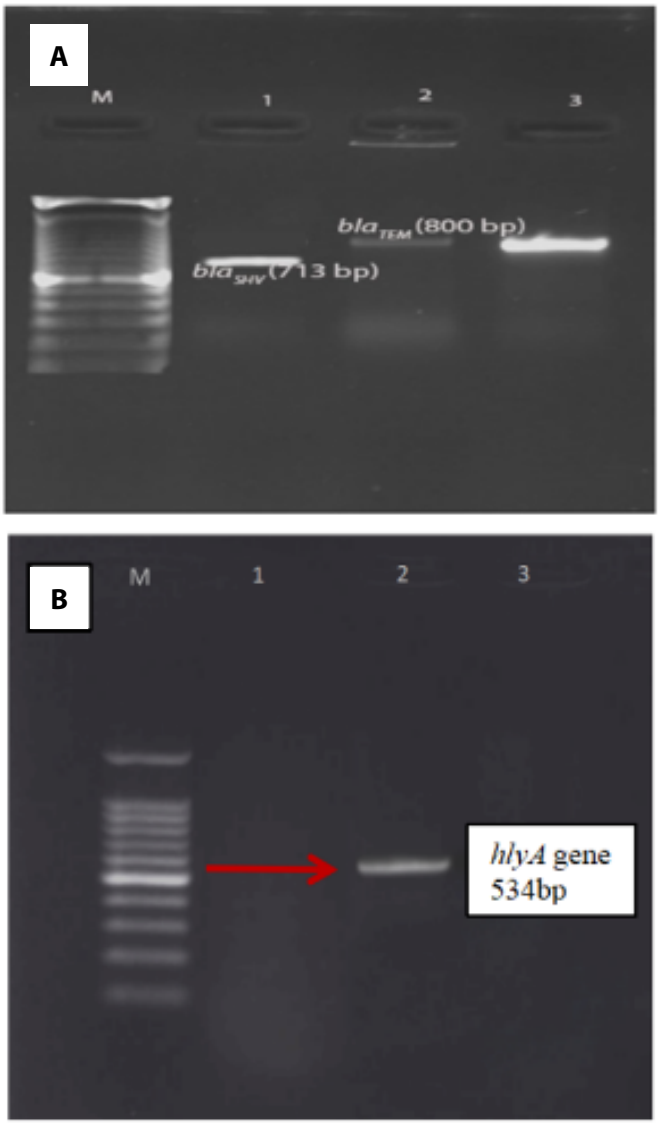


Figure 8. Detection of antibiotic resistance genes (a) and virulence gene (b) in plastic-associated bacterial isolates. Representative PCR amplification results showing the presence of *bla*TEM, *bla*SHV (β-lactamase resistance genes), and *hlyA* (hemolysin virulence gene). Bands corresponding to expected amplicon sizes confirm the presence of these genes in selected isolates.

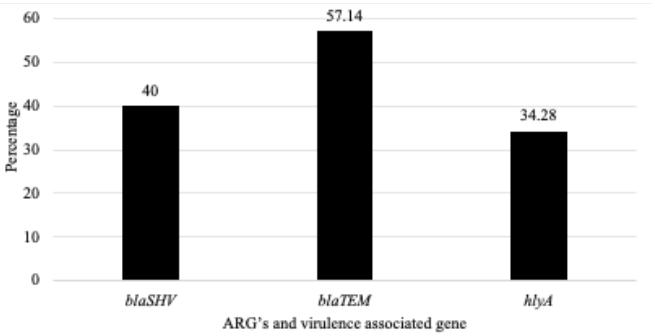


Figure 9. Prevalence of antibiotic resistance (*bla*TEM, *bla*SHV) and virulence (*hlyA*) genes among 35 biofilm forming bacterial isolates from plastic samples encountered in Antarctica

Antibiotic resistant genes and virulence gene among bacterial isolates

A total of 35 biofilm forming bacterial isolates were screened by PCR for the presence of antibiotic resistance genes *bla*TEM, *bla*SHV and virulence gene*hlyA*. Representative PCR amplification products are shown in Figure 8. The *bla*TEM, *bla*SHV, and *hlyA* genes were detected in 20 (57.14%) 14 (40%) and 12 (34.28%) isolates respectively (Figure 9). Some bacterial isolates harbored more than one target gene. The distribution of the detected genes among the bacterial isolates is summarized in Table S3.

Statistical analysis

Statistical analysis revealed no significant the relationship between biofilm forming category and the MAR index was assessed using Spearman's rank correlation, which showed a weak negative correlation (rho=-0.063, p=0.720). Furthermore, no statistically significant association was observed between biofilm forming category and MDR Based on chi-square test (p=0.994).

Discussion

This study investigated biofilm forming bacteria associated with macroplastic samples collected from the Antarctic environment and examined the prevalence of antibiotic resistance among the recovered biofilm forming bacterial isolates. A total of two macroplastics samples, approximately 5.5 cm and 7 cm in size were collected near the Indian Antarctic research station Maitri, as part of the 39th Indian Scientific Expedition to Antarctica (39th ISEA). FT-IR spectroscopy identified that the samples were PE and PP. Several studies, including those by Aves *et al*. (2022) and Jones-Williams *et al*. (2025) have reported the presence of these polymers in Antarctic environments. Consistent with previous reports, our study findings corroborate the widespread occurrence of PP and PE in Antarctic continent, further supporting the growing body of evidence that plastic pollution is persistent and widespread in this remote polar environment. Their widespread occurrence may be attributed to their extensive global use, durability, waste discharge from research stations and ships, and ability to undergo long range atmospheric and oceanic transport from lower latitudes. These findings are consistent with previous observations (Waller *et al*, 2017; Suraria *et al*, 2020). Considering that the macroplastic samples analyzed in the present study were collected in the vicinity of Maitri Station, local inputs associated with research station activities cannot be excluded. However, because the origin of the individual plastic items was not investigated, the relative contribution of local sources and long range oceanic or atmospheric transport could not be determined.

The SEM observations revealed dense bacterial colonization, and distinct weathering features such as pitting and cracks on plastic surfaces. Comparable findings have been reported in Antarctic environments, where biofilm formation is often accompanied by unique mechanical alterations, including abrasion marks and embedded mineral particles resulting from ice action and freeze thaw cycles (Laganà *et al*, 2019; Monràs-Riera *et al*, 2024; Lacerda *et al*, 2025). These parallels highlights that the biofilm formation is a universal characteristic of the plastisphere, but local environmental conditions strongly influence the morphology of microbial communities and their interaction with polymer surface.

The slightly higher bacterial load (3.3 x 10^5 CFU/ ml) on PE in the present study may reflect difference in microbial colonization associated with polymer type, consistent with findings from sub-Antarctic plastisphere studies (Arias-Andres *et al*, 2018). The recovery of 53 morphologically distinct bacterial strains isolated from the plastic samples highlights the diversity of bacterial communities associated with Antarctic plastic debris. The representative bacterial taxa identified in the present study, including *Acinetobacter baumannii*, *Planococcus maritimus*, *Priesta aryabhattai* and *Bacillus cereus*. Members of the genera *Acinetobacter*, *Planococcus*, *Priesta* and *Bacillus* have previously been reported from various Antarctic regions, demonstrating their ability to survive under cold and nutrient limited conditions (Sinha and Krishnan, 2013; See *et al*, 2017; Kang *et al*, 2023; Pazos *et al*, 2025).

Out of the 53 bacterial isolates 66% of them were biofilm producers (16.98% weak, 28.30% moderate, 20.75% strong biofilm formers). The formation of biofilms on macroplastics observed in the present study suggests that plastic debris provide a suitable microhabitat for microbial colonization in Antarctic plastics. Recent reviews of plastisphere biofilms across aquatic ecosystems emphasized that environmental factors and polymer characteristics influence microbial community assembly and biofilm development (Bocci *et al*, 2024). The persistence of such biofilms on plastics, even in remote polar ecosystems, may have important ecological implications, by enhancing bacterial survival and providing suitable conditions for the persistence and dissemination of antibiotic resistance genes.

In the present study, several biofilm forming bacteria exhibited resistance against several antibiotics, including cephalosporins, quinolones, and β-lactams (ampicillin, carbenicillin, etc.). These results are in tune with the observation of Laganà *et al*. (2019), who reported that bacterial isolates from a polystyrene piece were capable of biofilm formation and displayed multidrug resistance.

In our study, 48.57% of the bacterial isolates were multidrug resistant, with MAR indices ranging from 0.07 to 0.78. Notably, several isolates exhibited MAR indices ≥ 0.2, suggesting exposure to elevated antibiotic contamination or selective pressure. Similar findings (Laganà *et al*, 2019; Hwengwere, 2022 and Caruso *et al*, 2024), who reported biofilm-forming bacteria from Antarctic plastics with resistance to multiple antibiotic classes. Previous studies reported that plastic surfaces can adsorb antibiotics, with adsorption varying among polymer types and increasing after polymer ageing (Anbarani *et al*, 2020). Although antibiotic adsorption was not investigated in the present study, the detection of biofilm forming multidrug resistant bacteria on PP and PE suggests that plastic debris may provide favourable micro habits for the persistence of antimicrobial bacteria. Consistent with this, several bacterial isolates recovered in the present study exhibited resistance to cephalosporins, quinolones and β-lactam antibiotics.

Furthermore, several isolates exhibited the presence of antibiotic resistance genes *bla*$_{TEM}$, *bla*$_{SHV}$ and the virulence gene *hlyA*, indicating the occurrence of clinically relevant genetic determinants in plastic associated bacteria. Among the screened genes, *bla*$_{TEM}$ was the most prevalent, detected in 57.14% of isolates, followed by *bla*$_{SHV}$ in 40%, and *hlyA* in 34.28%. Similar observations were reported by Pazos *et al*. (2025), who identified antibiotic resistance gens in Antarctic *Acinetobacter baumannii* isolates. Likewise, earlier reports have shown that plastic debris in polar environments serves as a habitat for diverse bacterial communities, including potentially pathogenic bacteria carrying antibiotic resistance and virulence determinants (Lacerda *et al*, 2019; Beans, 2023; Zhai *et al*, 2023; Caruso *et al*, 2024). The higher prevalence of *bla*$_{TEM}$ compared to *bla*$_{SHV}$ aligns with global reports describing the widespread dissemination of *bla*$_{TEM}$-type extended-spectrum β-lactamases, especially among *Enterobacteriaceae* (Paterson and Bonomo, 2005; Rawat and Nair, 2010).

Although, only two macroplastic items were recovered during the sampling period, their occurrence in a remote Antarctic environment is ecologically significant. The detection of biofilm forming and multidrug resistant bacteria associated with these plastics demonstrates that even limited plastic debris can provide suitable substrates for microbial colonization and persistence in polar ecosystems. Future investigations incorporating surrounding environmental matrices such as sediment and water would provide essential ecological context and further clarify the role of plastic debris in shaping microbial communities and antimicrobial resistance in polar environments. Additionally, the findings of the present study highlight need for strengthened environmental management and bio safety practices in Antarctic research regions. Measures such as decontamination of scientific equipment, effective treatment of waste water produced during research station activities, responsible management of plastic waste, and long-term monitoring of plastic associated microbial communities and antibiotic resistance genes would help to minimize the introduction and persistence of antibiotic-resistant bacteria in Antarctic ecosystems. Collectively, these strategies contribute to preserving the ecological integrity of the Antarctica.

Conclusion

This study demonstrates that plastics in the Antarctic marine environment serve as substratum for diverse microbial communities, including bacteria capable of biofilm formation and exhibiting multidrug resistance. FT-IR analysis characterized the polymer types (polyethylene and polypropylene) present, while SEM imaging revealed extensive microbial colonization on these plastics. Notably, 48.57% of the bacterial isolates displayed multidrug resistance, indicating that macroplastic debris may serve as a reservoir for potentially pathogenic MDR microorganisms, even in remote Polar regions. These results highlight significant ecological and public health concerns and emphasize the urgent need for continuous monitoring and the development of strategies to mitigate the spread of antimicrobial resistance through environmental pathways to vulnerable polar ecosystems.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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