Single-Use Polyethylene terephthalate Bottles derived Nanoplastics Propagate Antibiotic Resistance in Bacteria via Transformation and Outer Membrane Vesicles Secretion

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1. Information S1 : Cell Lysis

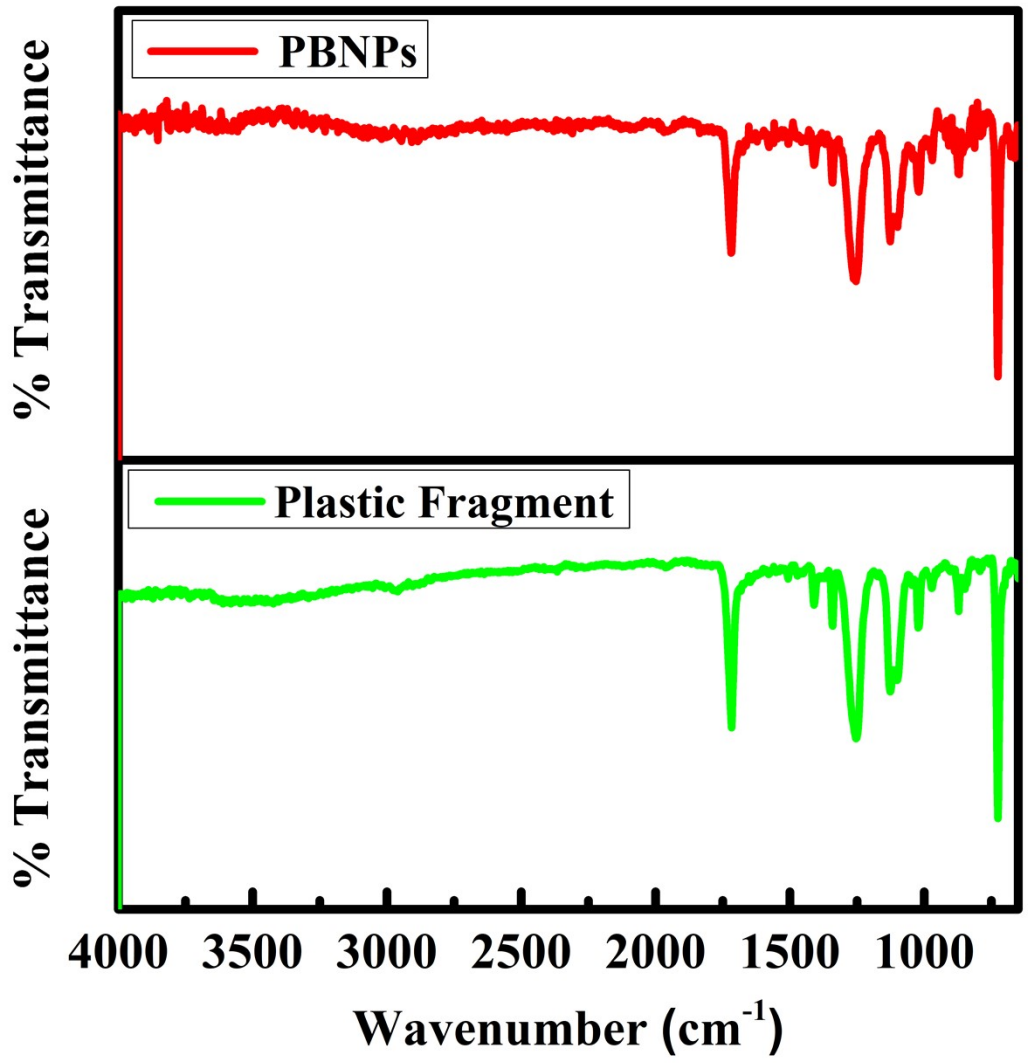
Bacterial cell lysis was carried out using sonication. The cells were pelleted through centrifugation at 8000 RPM for 10 minute and then pellet washed with phosphate-buffered saline (PBS). Then this pellet was lysed by probe sonication (15 cycles, each cycles of 30 sec. after 10 sec interval). To minimize heat generation, all samples were kept on ice. the sonicated samples were centrifuged at 10000 RPM, 20 min., 4^{o}C to separate cellular debris from the lysate. The supernatant, containing soluble cellular components, was collected and stored at -80°C for for biochemical assays.

2. Information S2: Protein Quantification

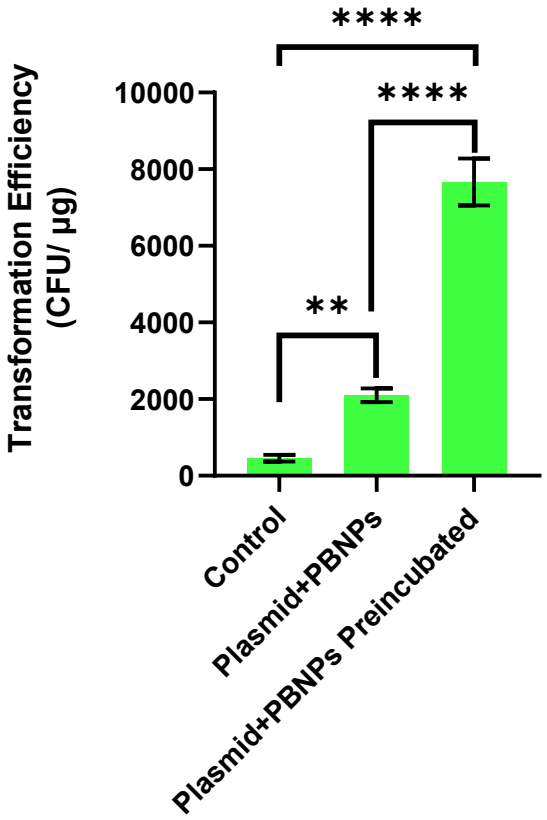
Protein quantification was conducted using the Lowry method. In this approach, the Folin–Ciocalteu reagent was diluted in a 1:2 ratio with ultra-pure water, and 0.5 mL of the resulting diluted reagent was introduced to 1.0 mL of the sample. The sample had been previously mixed with 5.0 mL of reactive "C," composed of 50 volumes of reactive "A" (2.0% Na_2CO_3 + 0.1 N NaOH) and 1 volume of reactive "B" (a combination of 1/2 volume of 1% $CuSO_4 \cdot 5H_2O$ and 1/2 volume of 2.0% $C_4H_4NaO_6 \cdot 4H_2O$) and incubated for 10 min. Following the addition of each reagent, the samples were stirred for 2 seconds using a test tube stirrer. Absorbance was then measured at 750 nm, 30 minutes after initiating the chemical reaction at room temperature.

3. Information S3: Plasmid Isolation

For crude isolation of all plasmids, the Alkaline lysis protocol as mentioned in the Molecular Cloning by Sambrook and Russell was followed. Briefly, 5 mL of overnight grown culture was pelleted and completely resuspended in 100 µL of ice-cold solution-I. To this, 200 µL of the freshly prepared solution-II was added and mixed thoroughly, followed by the addition of 150 µL of ice-cold solution-III. Lysates thus obtained were spun at 12,000 × g for 10 min, and the supernatant was transferred to a fresh tube. For the plasmid precipitation, 0.7 volumes of isopropanol were added to the supernatant. The mixture was allowed to stand at room temperature for 5 min and centrifuged at the maximum speed for 3 min. The pellet was washed once with 1 mL of 70% ethanol, removed residual ethanol, and incubated tubes (cap-opened) at 37 °C till the ethanol evaporated. The dried plasmid was resuspended in TE-RNase [1].



Supplementary Figure 1: FTIR spectra of plastic bottle fragments and PBNPs synthesized from them.



Supplementary Figure 2: Transformation efficiency

1. T. Sajeev, A. K. Panda, J. Kawadkar, D. Bindra, P. A. Joshi, G. Joshi, H. V. Singh and R. K. Mishra, in *Methods in Enzymology*, Elsevier, 2022, vol. 675, pp. 397-424.