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Abstract

Droplet-Based Technology for Studying the Phenotypic Effect of Microplastics on Antimicrobial Resistance †

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Plastic pollution is a global emergency [1,2]. One key problem is that microplastics (MPs) (1 µm–5 mm) [3] and nanoplastics (NPs) (≤1 µm) [4] enhance the already severe threat of antimicrobial resistance (AMR) by providing a micro-environment termed the "plastisphere" for bacteria to form biofilms [5,6]. However, exact knowledge of the severity of the plastisphere and its impact on AMR is currently still scarce [7]. Here, we show how droplet-based technology can be used to study the potential phenotypic effect of MPs on AMR. For this we used (i) polydisperse water-in-oil droplets generated via vortexing, (ii) GFB-labelled *Escherichia coli* JEK 1036 as our study object, (iii) cefotaxime as the test antibiotic, and (iv) 10 µm carboxylated polystyrene microspheres (PS). In parallel, we encapsulated single cells of *E. coli* into droplets with different concentrations of cefotaxime and with or without PS. After overnight incubation at 37 °C, we imaged droplets as a monolayer via confocal microscopy and analyzed droplets via Software Ilastik [8], CellProfiler™ [9] and EasyFlow [10]. Our results show that *E. coli*'s minimal inhibitory concentration (MIC) shifts slightly towards a higher cefotaxime concentration when PS is present in droplets. Image analysis of *E. coli* growth patterns in individual droplets illustrates that *E. coli* tends to clump together in droplets with PS, versus exhibiting an evenly distributed growth pattern in droplets without PS. In conclusion, we see that PS in droplets might enhance the MIC of *E. coli* resistance against cefotaxime. This possible enhanced resistance may be related to the observed tendency for clumping (indication of biofilm formation) of *E. coli* when PS is present. Droplet-based technology is thus a suitable tool for studying the phenotypic effect of MPs on AMR. Further experiments with different antibiotics and MP types and sizes will shed more light on the interesting and worrying tendency of MPs to potentially enhance AMR that was found in this study.

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References

1. Veidis, E.M.; LaBeaud, A.D.; Phillips, A.A.; Barry, M. Tackling the Ubiquity of Plastic Waste for Human and Planetary Health. *Am. J. Trop. Med. Hyg.* **2022**, *106*, 12. [CrossRef] [PubMed]
2. Brahney, J.; Mahowald, N.; Prank, M.; Cornwell, G.; Klimont, Z.; Matsui, H.; Prather, K.A. Constraining the atmospheric limb of the plastic cycle. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2020719118. [CrossRef] [PubMed]
3. Frias, J.P.G.L.; Nash, R. Microplastics: Finding a consensus on the definition. *Mar. Pollut. Bull.* **2019**, *138*, 145–147. [CrossRef] [PubMed]
4. Gigault, J.; ter Halle, A.; Baudrimont, M.; Pascal, P.-Y.; Gauffre, F.; Phi, T.-L.; El Hadri, H.; Grassl, B.; Reynaud, S. Current opinion: What is a nanoplastic? *Environ. Pollut.* **2018**, *235*, 1030–1034. [CrossRef] [PubMed]
5. Zhang, Y.; Lu, J.; Wu, J.; Wang, J.; Luo, Y. Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system. *Ecotoxicol. Environ. Saf.* **2020**, *187*, 109852. [CrossRef] [PubMed]
6. Imran, M.; Das, K.R.; Naik, M.M. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat. *Chemosphere* **2019**, *215*, 846–857. [CrossRef] [PubMed]
7. Bartkova, S.; Kahru, A.; Heinlaan, M.; Scheler, O. Techniques Used for Analyzing Microplastics, Antimicrobial Resistance and Microbial Community Composition: A Mini-Review. *Front. Microbiol.* **2021**, *12*, 413. [CrossRef] [PubMed]
8. Berg, S.; Kutra, D.; Kroeger, T.; Straehle, C.N.; Kausler, B.X.; Haubold, C.; Schiegg, M.; Ales, J.; Beier, T.; Rudy, M.; et al. ilastik: Interactive machine learning for (bio)image analysis. *Nat. Methods* **2019**, *16*, 1226–1232. [CrossRef] [PubMed]
9. Carpenter, A.E.; Jones, T.R.; Lamprecht, M.R.; Clarke, C.; Kang, I.H.; Friman, O.; Guertin, D.A.; Chang, J.H.; Lindquist, R.A.; Moffat, J.; et al. CellProfiler: Image analysis software for identifying and quantifying cell phenotypes. *Genome Biol.* **2006**, *7*, R100. [CrossRef] [PubMed]
10. Sanka, I.; Bartkova, S.; Pata, P.; Ernits, M.; Meinberg, M.M.; Agu, N.; Aruoja, V.; Smolander, O.-P.; Scheler, O. User-friendly analysis of droplet array images. *Anal. Chim. Acta* **2023**, *1272*, 341–397. [CrossRef] [PubMed]