eDNA assessment of potentially pathogenic bacteria in the Gulf of Naples

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Coastal marine ecosystems are increasingly threatened by anthropogenic activities, including the discharge of human sewage and agricultural runoff through wastewater disposal systems. In addition, climate change–driven variations in salinity, temperature, nutrient availability, and light can modulate survival and proliferation of potential pathogens, increasing the risk of transmission to humans and marine organisms. Monitoring coastal areas is therefore crucial for detecting shifts in microbial communities, especially through the integration of omics approaches.

In this study, we used 16S rRNA metabarcoding to detect potential pathogens in the Gulf of Naples. To this aim, we analyzed the dataset generated in 2024 through monthly sampling at the MareChiara LTER site (LTER-MC) in the framework of the activities of the Italian Omics Observatory Network and the NEREA initiative (www.nerea-observatory.org). Fractionated samples were collected filtering sequentially through 0.2, 3 and 20 µm filters. Additional net samples (20-200 µm and 200-2000 µm fractions) were also analyzed. From a total of 59 samples, we identified 17 genera potentially containing pathogenic species, corresponding to 523 ASVs.

Multivariate analyses and LEfSe (Linear Discriminant Analysis Effect Size) analysis showed that distinct genera were associated with different seasons and size fractions. *Vibrio* spp. and Enterobacterales were identified as summer bioindicators, while *Pseudomonas*, *Streptococcus*, and *Clostridium* characterized winter samples. No potential pathogenic genus was statistically associated with the free-living fraction (0.2 µm), suggesting that these especially thrive when associated with particles (living or non-living), and not free in the seawater.

Overall, these findings highlight the combined influence of seasonal variability and particle association in shaping the distribution of potentially pathogenic bacteria in coastal waters of the Gulf of Naples, emphasizing the importance of their traceability in long-term monitoring efforts to assess marine ecosystem health and its direct implications for human health.