

From Frozen Desolation to Verdant Wetlands: A Microbial Voyage from the Antarctic to the Danube Delta Biosphere Reserve

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Abstract: As global warming intensifies, polar ecosystems face potential shifts in biodiversity and stability, while temperate protected areas such as the Danube Delta (DD) face pressures from anthropogenic pollution and antimicrobial resistance (AMR) dissemination. Understanding how these contrasting environments act as microbial reservoirs is critical for assessing global health risks. Here, we propose a comparative shotgun metagenomic analysis of microbial communities from Antarctic ice cores (n=18) and DD water samples (n=8), with a focus on the environmental reservoir of AMR within the DD biosphere reserve. Ice samples from the Barton Peninsula (Antarctic South Shetland Islands) and water samples from the DD were processed using the DNeasy PowerWater Kit, sequenced by Illumina MiSeq/NextSeq platforms, and analyzed with a standardized bioinformatics pipeline. Quality control (HTStream, Trimmomatic), de novo assembly (MEGAHIT), taxonomical profiling (Metaxa2), and functional profiling of AMR genes and mobile genetic elements (MGEs, DIAMOND) were performed. DD samples displayed a homogenous microbiota dominated by Polynucleobacter sp., Limnohabitans sp., and Delftia sp., taxa typically linked to aquatic ecosystems, pollutant biodegradation, and heavy metal detoxification. Importantly, DD revealed a low but non-negligible diversity of ARGs, including qacEΔ1 and sul1, alongside aminoglycoside and metal resistance determinants. MGEs were also identified, including insertion sequences (IS91) and Synechococcus-associated phages, some with ≥90% identity. These findings highlight the DD as a sensitive aquatic reservoir of AMR and MGEs within a protected European biodiversity hotspot. By contrast, Antarctic samples showed broader microbial diversity, including genera such as Arthrobacter, Clostridium, Rheinheimera, Novosphingobium, Synechococcus, and Streptomyces, indicative of extreme and marine-associated conditions. Antarctic communities harbored distinct MGEs (ICEs, transposons Tn6228 and Tn4371), linked to heavy metals, antibiotics, and virulence factors. Together, these results emphasize the unique vulnerability of the DD to AMR dissemination, in contrast to the natural resilience and extremophilic adaptations of Antarctic microbiota.

Keywords: Antarctic vs. Danube Delta; Shotgun Metagenomics; AMR Reservoirs; Mobile Genetic Elements; Microbial Community Contrast.

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