## ADVANCES IN MICROBIOTA KNOWLEDGE AND NGS TECHNOLOGIES: PERSPECTIVES FOR SURVEILLANCE IN RECREATIONAL WATERS

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Massive sequencing is widely diffusing in several biomedical contexts opening new chapters in microbiota knowledge and suggesting new applications for public health purposes. The human microbiota project represents a milestone in the field, showing the impressive role of the many beneficial species respect to the rare and devastating impact of single pathogens. Environmental biodiversity is also being studied in different matrices, such as water, air, soils, sediments and surfaces from different materials, suggesting concrete applications in different areas of public health. Despite the large attention from basic and applied sciences, this approach is not yet diffused in hygiene and prevention, including the specific world of the recreational and wellness spaces, such as pools and SPA. The equipment costs and know-how availability are not the only limiting factors; several cultural and technological restrictions play a major role: the novelty itself, the lack of guidelines and -most of all- the bioinformatics bottleneck. Nevertheless, bioinformatics platforms are available including one dedicated to SPA waters (www.mfatlas.it), showing the extraordinary biodiversity of hot springs and the potentials for pools and wellness applications (ICSPS2017).

Regarding the biological component present in recreational waters, several factors can influence its final structure in pools, such as the source properties, the development of biofilms along the pipeline, the presence of anthropic or environmental contaminations. Even if classical methods represent the gold standard in surveillance, the massive sequencing approach can provide additional information, but requires dedicated protocols. The fundamental step is sampling of the microflora DNA (mfDNA). Innovative tools and appropriate protocols are already available or under implementation. Then, Next Generation Sequencing (NGS) analysis can be performed by different procedures to the final aim of evaluating biodiversity and defining a microbial signature. This approach can be considered semi-quantitative and informative for detection of unculturable or unknown species, assessment of hygiene indicators, acquisition of data on disinfection effectiveness or on the presence of pathogens. In this scenario, the water microbiota itself is going to represent a novel marker to asses water quality and safety.

The application of microbiota analysis in public health and surveillance of recreational waters is going to be a promising approach, but it is still limited by the lack of software-driven databases, even if several user-friendly toolboxes already are available. Complex microflora -both planktonic and benthonic- can be studied by sequencing the mfDNA and considering the NGS output as a kind of metagenome corresponding to a ecosystem. Swimming pool and SPA waters represent a very heterogeneous matrix and an interesting study-model along the whole water-supply chain: from reservoirs and water springs, through pipelines and treatments, to pools and finally wastewaters. The analysis of the microflora composition through the study of its mfDNA provides information on water identity at its source, quality of system management, interference of disinfectants and other treatments, identification of critical points for preventing outbreaks. The whole of literature and experimental data allowed the definition of collaborative networks and projects aimed to validate mfDNA as a candidate indicator for public health, both in recreational waters and other matrices.