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Genetic diversity of norovirus Genogroups I and II in untreated wastewater in Rome Italy: a five-year monitoring study

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Introduction: Wastewater-based epidemiology applied to virology is an epidemiological complementary tool in the field of infectious disease surveillance to reveal viral circulation in the population, whether in symptomatic, asymptomatic, or preclinical states of disease. Human viruses released into feces and urine make their way into the environment by excretion or secretion of bodily fluids or skin cells. Among them, Human Norovirus (HNoV), belonging to the *Caliciviridae* viral family, is the most common etiological agent responsible of acute gastroenteritis in humans of all age. It is the leading cause of non-bacterial acute gastroenteritis (AGE) worldwide, causing approximately 18% of AGE and 212,000 deaths every year. Currently, HNoVs are divided into 10 genogroups and 49 genotypes, of which GI, GII, and GIV are known to infect humans, The aim of this study was to provide data on the genetic diversity of GI and GII HNoVs over a five-years period, testing urban sewage samples.

Materials and Methods: Between 2017 and 2021 a total of 260 sewage samples were collected from four wastewater treatment plants in Rome. Sewage concentration was performed by the two-phase polyethylene glycol (PEG), dextran separation method. Viral nucleic acids were extracted using the NucliSENS MiniMag extraction system (bioMerieux). All samples were analyzed by nested RT-PCR targeting region C (capsid gene). The PCR amplicons were pooled per year and analyzed by NGS carried out on MiSeq II sequencer (Illumina). Subsequent bioinformatic analysis was carried on with a similarity-based approach.

Results: Seven different GI capsid genotypes (GI.1, GI.2, GI.3, GI.4, GI.5, GI.6 and GI.7) were detected, and the most abundant were GI.1, GI.2 and GI.4. Among GII, thirteen genotypes (GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.9, GII.10, GII.12, GII.13, GII.16, GII.17 and GII.21) were reported. During the first two years, GII.2 and GII.4 were prevalent until decreasing, in 2019, for the appearance of GII.5, GII.6, GII.7 and GII.12. Across the years, GII.3, GII.13, and GII.17 were always present, and the GII.4 Sydney was the prevalent variant. This strain was first detected in Australia in 2012 and rapidly became the major epidemic strain in Europe, America and Asia in 2012–2013.

Discussion and Conclusion: Next-generation sequencing proved to be an effective strategy for HNoV genotyping in wastewater samples. A significant variety of major and minor norovirus GI and GII genotypes was reported in wastewater in Rome. Complementary data obtained from both clinical and environmental samples can be an effective strategy for understanding the diversity and evolutionary dynamics of HNoVs and infere the epidemiological status of the population.