

## Exploring the Nasopharyngeal Tract Phageome Dynamics and its correlation with Disease Severity and Patient Age Across Three COVID-19 Waves

Folliero Veronica<sup>1</sup>, Ferravante Carlo<sup>1</sup>, Arslan-Gatz Berin S.<sup>1</sup>, Dell'Annunziata Federica<sup>1</sup>, Palumbo Domenico<sup>1</sup>, Lamberti Jessica<sup>1</sup>, Alexandrova Elena<sup>1</sup>, Di Rosa Domenico<sup>1</sup>, Strianese Oriana<sup>2</sup>, Giordano Alessandro<sup>1</sup>, Palo Luigi<sup>1</sup>, Giurato Giorgio<sup>1</sup>, Salzano Francesco A.<sup>1</sup>, Galdiero Massimiliano<sup>3</sup>, Weisz Alessandro<sup>1</sup>, Franci Gianluigi<sup>1</sup>, Rizzo Francesca<sup>1</sup>

1. Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, Baronissi, Italy

2. Genome Research Center for Health - CRGS, Campus of Medicine - University of Salerno, Baronissi, Italy

3. Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy.

**Introduction-Aim.** The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has stimulated scientific investigations into the influence of the microbiota on disease severity, patient age cohorts and the temporal progression of the disease. An often-overlooked aspect of the microbiome is the role played by bacteriophages. This study aims to systematically explore phage profiles in the context of the three conditions mentioned above. **Materials and Methods.** This study involved 55 individuals positive for SARS-CoV-2 in the Campania region. Nasopharyngeal swabs were systematically collected during three distinct SARS-CoV-2 epidemics in Italy: March-May 2020 (n.25); September-November 2020 (n.25); January-February 2021 (n.5). The cohort was stratified by symptom severity, with 39 cases classified as non-severe, 6 as moderate, and 10 as severe. The age spectrum of enrolled patients ranged from 8 to 91 years. RNA extraction was performed using the ELITeInGenius system, followed by sequencing of RNA samples on the NextSeq 500 platform. Phage abundances were subsequently analyzed using HOME-BIO 15 software. **Results.** In the analysis of 55 nasopharyngeal swabs from COVID-19 patients, a total of 6 phage families were identified. *Siphoviridae* emerged as the most widespread family, followed closely by *Myoviridae*. Examination of dynamics across the three pandemic waves revealed that *Peduvirinae* were more abundant in wave I than in wave II, a trend mirrored by *Autographiviridae* and *Microviridae*. Similarly, the first period showed higher frequencies of *Peduvirinae*, *Autographiviridae* and *Microviridae* than the third period. Notably, these phage families were more prevalent in samples from patients with severe symptoms compared to those with non-severe conditions. *Siphoviridae*, *Myoviridae*, and *Microviridae* were less abundant in patients without symptoms compared to the severe group. Regarding age, significant differences were observed only in 2 phage families: *Autographiviridae* and *Siphoviridae*, which were less abundant in patients aged 41–59 years compared to all other age groups. **Conclusion.** In the course of SARS-CoV-2 infection, notable alterations occur in the composition of the phageome within the nasopharyngeal tract. These discernible changes offer valuable insights that can inform the development of more effective management strategies in the ongoing battle against COVID-19.