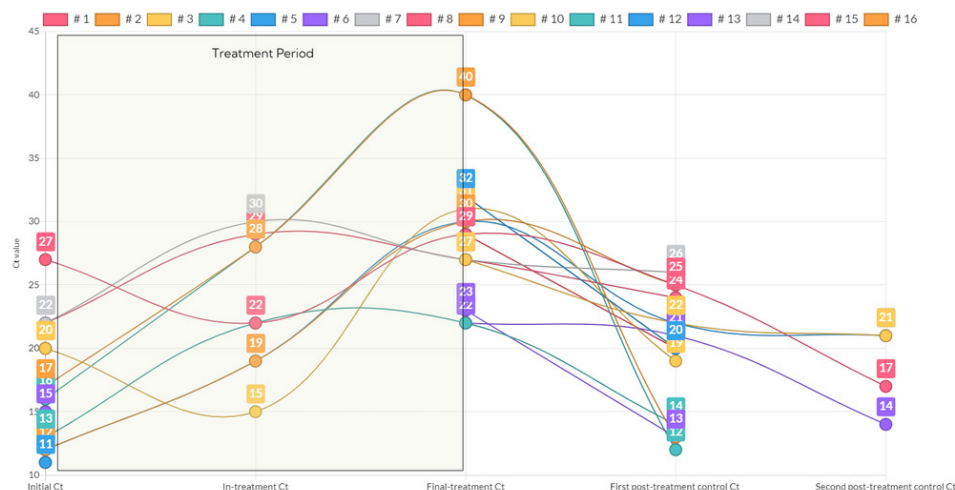


Figure 1. Viral load dynamics measured by Ct values at various treatment timepoints and post-treatment



P0578 | 07815

## Nasopharyngeal microbiome in COVID-19 infection

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### Background

COVID-19 pandemic has triggered the need to understand in greater depth the mechanisms that regulate the entry and establishment of some respiratory viruses in the human body. Human microbiome has shown a key role in training and regulating the adaptive immune response to pathogens. Microbiome studies usually rely on sequencing the V3-V4 region of 16S rRNA gene or sequencing the whole metagenome content. The aim of this study was to evaluate the nasopharyngeal microbiota as potential predictor of the outcome of COVID-19, by whole 16S rRNA gene sequencing with Oxford Nanopore long reads technology.

### Methods

Nasopharyngeal swabs were collected at admission in our hospital and a year later from 127 COVID-19 positive patients. These samples were split in 2 experiments. Amplification of V1-V9 16S rRNA region and sequencing was performed using MinION (Oxford Nanopore Technologies). Bioinformatic analysis was carried out using a combination of in-house scripts, Microbiome Analyst web tool and Epi2me platform.

### Results

Experiment A: We compared samples collected at day 0 after admission from 31 patients who died from COVID-related complications and 26 more who survived more than one year. This experiment showed that certain genera like *Dolosigranulum*, *Veillonella* and *Actinomyces* were strongly related to the first group, while other clades (*Proteobacteria*, *Lawsonella*, *Achromobacter* and *Cutibacterium*) were closely linked to the second group of patients (figures 1 and 2). Experiment B (65 surviving patients): comparison among 130 samples collected at admission and 365 days after showed significant changes in the alpha and beta diversity in nasopharyngeal microbiota, perhaps due to a more general dysbiosis linked to viral infection (figure 3).

### Conclusions

The MinION long read approach has proven to be a valuable compromise between the accuracy of metagenomics and the cheapness of 16S rRNA high throughput studies. *Dolosigranulum*, *Veillonella* and *Actinomyces* colonization seems to be related to poor outcomes in SARS-Cov2 infection. Further studies will help to shed some light on the mechanisms underlying these results.

Figure 1. Heat phylogenetic tree showing clades enriched in samples whose outcome was: exitus (red) or survival (green)

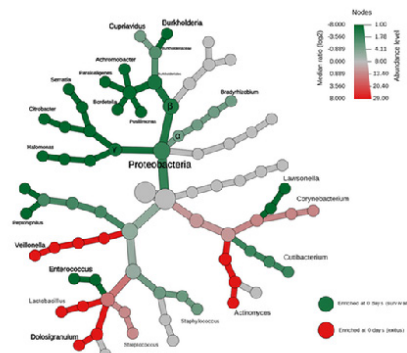


Figure 1. Heat phylogenetic tree showing clades enriched in samples whose outcome was: exitus (red) or survival (green). Enrichment analysis is shown in logarithmic scale of  $\log_2(N_{survivors}/N_{deceased})$ .

Figure 2. Clades enriched in surviving patients (green and purple) or exitus (orange).

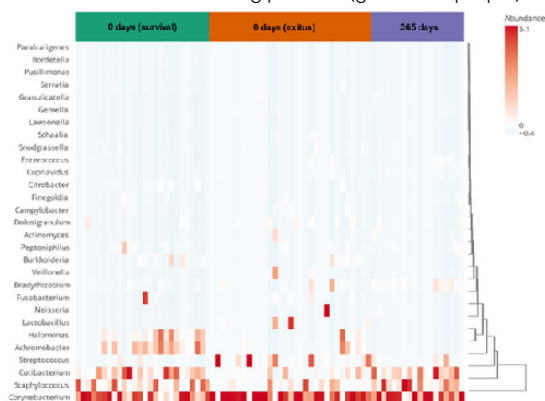


Figure 2. Clades enriched in surviving patients (green and purple) or exitus (orange). Most abundant clades (logarithmic scale as shown at top, right) are shaded in red, less abundant ones in white.

Figure 3. Alpha diversity decrease in nasopharyngeal swab samples between ICU admission and 1 year after.

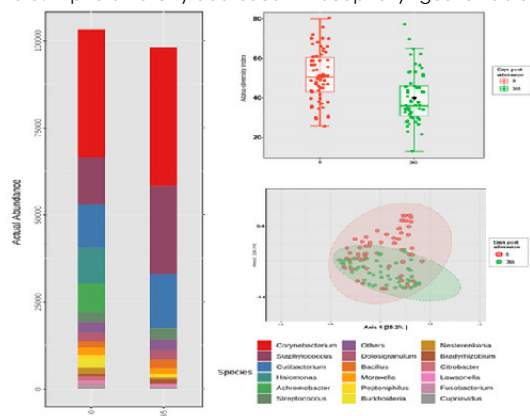


Figure 3 Alpha diversity decrease in nasopharyngeal swab samples between ICU admission and 1 year after. Panel left shows 20 most abundant genera (sum of total reads) grouped by sampling day: left 0 days after admission, right 365 days after. Colours represent genera (see legend, bottom right). Right panel top shows total alpha diversity measurements per sample at 0 or 365 days after admission. Right centre panel shows PCoA for beta-diversity measurements of samples at 0 (red) or 365 days (green) after admission, beta-diversity between groups measured with Jaccard Index and comparison with PERMANOVA. Alpha diversity was measured as total OTUs with more than 20 reads (0.1%) found in each sample. Samples were randomly rarefied at 2000 reads.

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## Investigating the impact of vaccination and reinfection dynamics on the XBB variant of SARS-CoV-2 < < <

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### Background

The XBB recombinant lineage of SARS-CoV-2, noted for its antibody evasion capabilities, has shown increased transmissibility without a corresponding increase in disease severity. Our research explores the relationship between infection or reinfection with the XBB variant and the administration of the SARS-CoV-2 vaccine.

### Methods

We sequenced SARS-CoV-2 positive samples from both public and private laboratories in the 4th Regional Health Coordination of RS, collected during epidemiological weeks 47/2022 to 17/2023. Sequencing utilized MinION or Illumina iSeq100, with ARTIC nCoV-2019 or Dragen COVID protocols for consensus assembly. Clades and lineages were identified by Nextclade. Clinical and epidemiological data were sourced from DATASUS, e-SUS, and SIVEP GRIPE.

### Results

Of the 340 sequenced samples, 89 were identified as XBB or its sublineages. Of the patients infected with XBB, 97.7% recovered, and 2.3% died due to COVID-19 complications (Figure 1A). Only 41.6% had the full vaccine scheme. A significant portion (57.3%) experienced reinfection, having been previously vaccinated, which indicates hybrid immunity (Figure 1B). The median interval between the last vaccine dose and XBB infection varied across age groups: 403 days for ages 12-39, 442 days for ages 40-60, and 351 days for those over 60 (Figure 2). All infected children aged 0-11 were unvaccinated. Genetic analysis revealed that XBB, a derivative of the BA.2.10.1 and BA.2.75 lineages, harbored 12 unique mutations, predominantly in the S gene (Figure 3). In the RS region, XBB lacked the characteristic mutations, namely G959P (ORF1b), E180V (S gene), K478R (S gene), F486S (S gene), R493Q (S gene), Q613H (S gene), and S84L (ORF8).

### Conclusions

Despite the high recovery rate in XBB cases, most individuals having received their last vaccine dose over a year, suggesting a potential waning of vaccine-induced immunity. The mutations in the S gene may affect the efficacy of current treatments and vaccines. Our findings highlight the importance of updated vaccinations and genomic surveillance in managing emerging SARS-CoV-2 variants.