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Metatranscriptomic Insights into Host-Microbiome Interactions Underlying Asymptomatic COVID-19 Cases

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Abstract:

Coronavirus disease 2019 (COVID-19) remains a major global health concern, with emerging evidence highlighting the role of the human microbiome in influencing disease severity. While extensive research has been conducted on COVID-19, studies examining host-pathogen interactions at the transcriptomic level remain limited. In this study, we investigated the metatranscriptomic profiles of forty nasopharyngeal samples collected from COVID-19 patients across different Bangladeshi cohorts. Sequencing data were processed to analyze taxonomic composition, microbial diversity, and antimicrobial resistance gene (ARG) patterns using multiple bioinformatic pipelines. COVID-19 positive and asymptomatic patients exhibited a higher abundance of pathogenic and multidrug-resistant bacteria, whereas COVID-19 negative individuals showed increased fungal diversity. Differential gene expression analysis revealed significant upregulation of immune response related genes, including pro-inflammatory cytokines, in COVID-19 positive cases. Notably, asymptomatic patients demonstrated reduced TLR4 expression, suggesting a potential reducing of innate immune activation, which may contribute to asymptomatic clinical outcomes. Additionally, functional enrichment highlighted active ARG expression in positive cases, indicating potential links between the respiratory microbiome and host immune modulation. These findings provide insights into the host-microbiome interplay underlying COVID-19 severity and highlight the need for further validation in larger, ethnically diverse cohorts with comprehensive clinical metadata.

Keywords: COVID-19, Metatranscriptomics, Immunological signaling, Differential gene expression, Transcriptome

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has created a catastrophic event throughout the world known as COVID-19 pandemic [1,2]. The first cluster of COVID-19 cases was identified in Wuhan, China, in late December 2019, where patients presented with symptoms of severe pneumonia. Since then, SARS-CoV-2 has been considered as one of the deadliest human pathogens after Spanish Flu [3,4]. However, fatality and prognosis of COVID-19 are influenced by several factors such as patients' age or preexisting comorbidities (diabetes, hypertension, asthma etc.) [5,6]. Vaccines have made it possible to reduce the severity of the disease, but emergence of such viral disease still remains a threat. Since the COVID-19 outbreak, there has been a significant increase in the collection of epidemiological, clinical, and immunological data. But the molecular mechanism underlying SARS-CoV-2 virus pathogenesis and prognosis was still not explored much [7,8]. This is because a thorough understanding of the pathogenesis of COVID-19 should enable the development of effective strategies to enhance the generation of effective anti-SARS-CoV-2 immune responses.

The respiratory microbiota consists of a wide range of bacteria and is distributed in the upper respiratory tract (URT). This microbial flora contains either commensals or pathobionts. Various investigations have shown that the severity of SARS-CoV-2 infection is likely to be influenced by the co-existing bacteria, fungi, and archaea in the respiratory tracts [9,10]. The SARS-CoV-2 virus enters the body by inhalation and binds to epithelial cells in the nasal cavity. It then replicates and migrates through the respiratory tract and gradually proceeds towards the lungs to initiate infection which in turn mounts a vigorous innate immune response for clearing up the virus [11]. So, it is believed that during this propagation and immune response, the microbiota residing in the respiratory airways may be altered, and inclusion of some of the pathobionts might alleviate the course and severity of the disease caused by SARS-CoV-2 virus [11].

Nowadays, Next-generation sequencing (NGS) approaches, such as metagenomics, is the widely used method for analyzing what kind of microbial population is present in a sample. But metagenomics mainly focuses on the genomic content and identification of microbes present within a community, while metatranscriptomics provides the diversity of the active genes within such community, their expression level profile and how these levels change with alterations in environmental conditions. Thus, to understand the role of respiratory microbiome in COVID-19 severity and to elucidate host gene expression with functional profiling metatranscriptomics approaches are preferable.

In this study, nasopharyngeal samples of different severity groups of COVID-19 patients were collected and subjected to sequencing to analyze both respiratory microbiome and differential gene expression and immunological signaling pathways of the host [12,13]. This information not only can be used for taxonomic characterization of known or novel microbes and host response analysis but also determines the functional profile of biological processes of normal and altered respiratory microbiome [13,14].

Results:

Taxonomic and microbial diversity analysis:

Sequencing yielded an average of 3.1 million paired-end reads per sample. Read distribution analysis revealed consistent coverage across coding regions in all samples, with comparable representation of 5′UTR and 3′UTR segments (S2). No sample showed a dominant accumulation of reads at the 3′ end or a depletion of 5′ transcript regions, indicating the absence of strong 5′–3′ degradation bias. The observed mild positional variation is consistent with expected Illumina RNA-seq library characteristics [15]. Ribosomal RNA depletion efficiency analysis showed only a small fraction of reads mapped to rRNA features (<0.5%, with a mean of 0.14% ± 0.10%), confirming effective depletion and high-quality library preparation. The percentage of rRNA across the samples is plotted in a barplot (S2).

Alignment to the human genome revealed that approximately 44% of these reads were of human origin, while the remaining 56% corresponded to microbial sequences. These microbial reads were subsequently used for all taxonomic analyses.

Alpha diversity measured using Shannon and Simpson Diversity metrics showed significant species diversity within the sample groups in terms of prokaryotes (Fig 1a). But in terms of Eukaryotes, the box and whisker plot show widespread distribution of species diversity for the severe group, whereas for the other three groups, the data were clustered (Fig 1d).
However, Bray–Curtis distance method Beta diversity analysis showed a separate cluster for Asymptomatic group in PCoA but the clusters of other three sample groups (Negative, Mild, Severe) overlapped for both the prokaryotes and eukaryotes (Fig 1b, 1e).

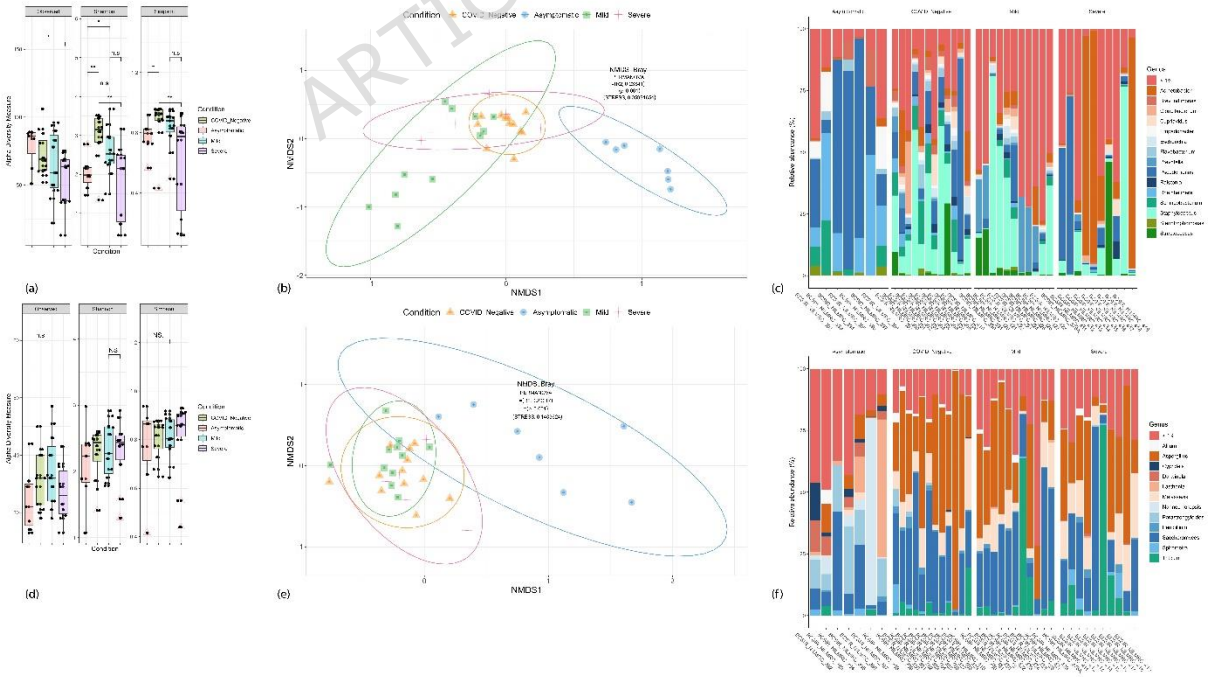

Fig 1: Alpha (a) and Beta (b) diversity indexes in Prokaryotes. Alpha (d) and Beta (e) diversity indexes in Eukaryotes. Genus level Relative abundance of four major study cohorts. Here, (c) Prokaryotes and (f) Eukaryotes.

The relative abundance analysis across the four major study groups suggested a higher presence of potentially pathogenic and multidrug-resistant bacterial strains among COVID-19 positive patients, regardless of disease severity (Fig. 1c, 1f). Additionally, COVID-19 positive samples appeared to show an increased fungal representation alongside SARS-CoV-2 infection.

Microbial metatranscriptomics and antimicrobial resistance analysis:

The diversity in microbial community delved into looking more closely at the condition of microbial metatranscriptomics in the samples. This analysis reveals the functions of microbial communities by checking the transcriptome of the microbiome in each environment.

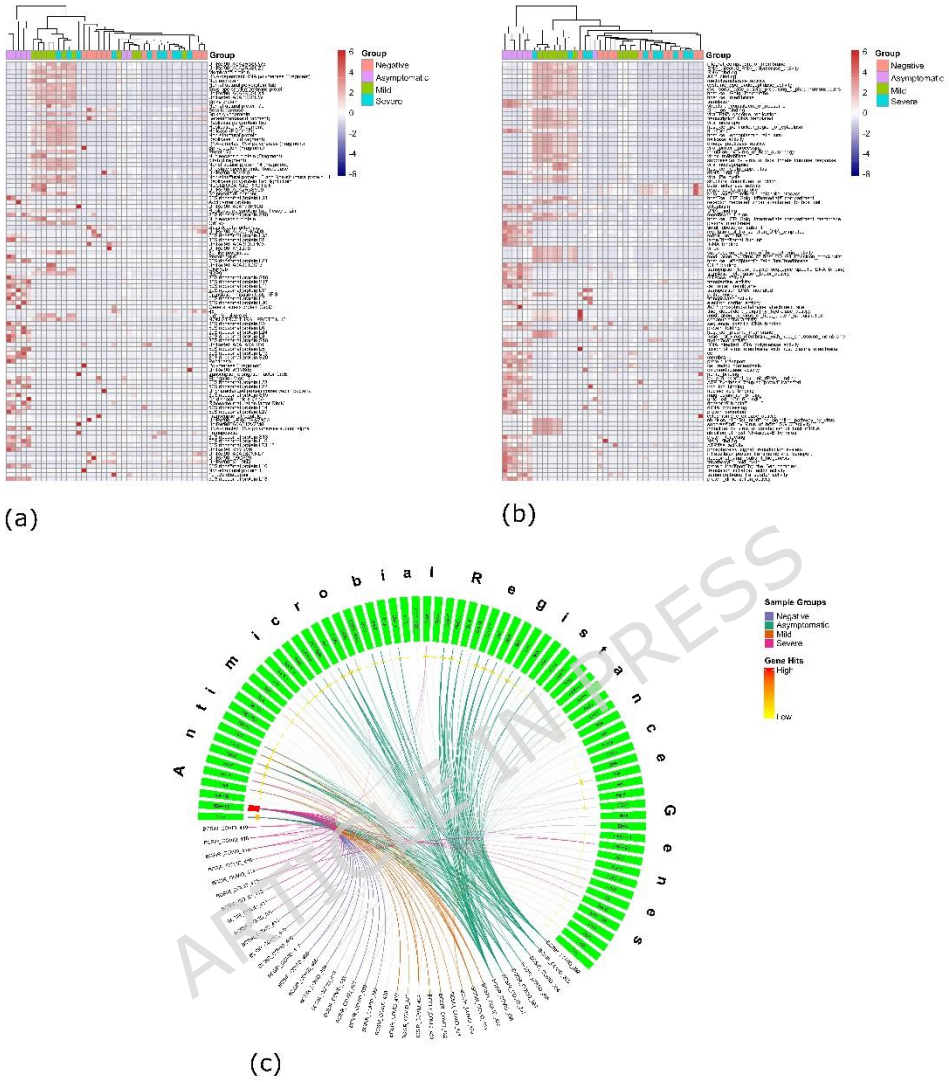

Fig 2: Here, (a) showing Gene Family abundance and (b) showing GO Term abundance in all the samples. Also, (c) showing a circos plot analysis of antibiotic resistance in asymptomatic positive patients visualizing the presence of different AMR genes across the isolates. A heatmap in the inner circle of the plot illustrates the frequency of each AMR gene across all isolates, with red indicating high frequency and yellow indicating low frequency.

Differences in gene expression patterns and functional activity were observed in different study cohorts of COVID-19 patients in terms of severity. Substantial up-regulation of translation machinery and ribosomal proteins, including multiple 50S and 30S ribosomal proteins, was observed in the microbial gene expression profile of asymptomatic patients (Fig 2a). Along with that, genes linked to porins, and cold shock proteins were also highly expressed. Moreover, Gene Ontology (GO) analysis revealed enrichment in molecular functions including DNA binding,

GTPase activity, and magnesium ion binding, as well as biological processes like translation elongation, protein transport, and the tricarboxylic acid cycle (Fig 2b).

Significant up-regulation of viral genes, such as replicase polyprotein 1AB, spike glycoprotein, and RNA-directed RNA polymerase, was observed in mild COVID-19 patients. The structural components of virions, host immune response regulation, and processes related to the viral life cycle were found to have enrichment, according to GO analysis.

Stress response proteins, such as Rubredoxin and class D beta-lactamase, were up-regulated in the microbial gene expression profile of severe COVID-19 patients. GO analysis showed inhibition of the host antiviral signal, modification of host protein ubiquitination, and enrichment in processes associated with the viral life cycle.

Genes related to beta-lactamase activity, cytochrome C oxidase activity, and aerobic respiration were all overexpressed in the negative group. GO enrichment reflected normal cellular activity in the absence of severe viral infection and was linked to host immunological suppression by viral processes, sensitivity to drugs, and mitochondrial function.

Analysis of antimicrobial resistance gene (ARG) profiles across the study cohorts revealed a markedly higher abundance and diversity of ARGs in the asymptomatic COVID-19 group compared to mild, severe, and negative cohorts. The asymptomatic COVID-19 cohort harbored a distinct resistome, enriched in genes conferring resistance to aminoglycosides, trimethoprim, phenicols, and rifamycins, which were largely absent in other severity groups. However, the β-lactamase gene *TEM1-D* was present across all cohorts and highlighted in red in the circos plot to denote its high abundance (Fig 2c).

To further explore the functional activity of the microbiome's resistome, a differential abundance analysis of ARGs was performed. This analysis revealed a distinct and statistically significant upregulation of multiple ARGs in the asymptomatic cohort compared to symptomatic (mild and severe) patients. In total, 22 ARGs were found to be significantly more abundant in asymptomatic individuals (*adjusted* $p < 0.05$; Supplementary File S2). Notably, the most upregulated genes included those conferring resistance to catalases (*CATA2*; $\log_2FC = 9.32$, p adj $= 1.73 \times 10^{-4}$) and macrolides (*MSRE*; $\log_2FC = 8.79$, p adj $= 3.82 \times 10^{-5}$; *MPHE*; $\log_2FC = 8.50$, p adj $= 1.37 \times 10^{-3}$). Additionally, genes conferring resistance to β-lactams (*PER-1*; $\log_2FC = 8.26$), sulfonamides (*SULII*; $\log_2FC = 8.13$), and aminoglycosides (*STRA*, *STRB*) were also highly expressed in the asymptomatic group.

COVID-19 associated host gene expression and gene enrichment:

The different transcriptional patterns of the Severe, Mild, Negative, and Asymptomatic groups may be clearly seen in the PCA plot (Fig 3). The biological significance of the transcriptome variations is shown by the clustering patterns and confidence ellipses, which show the variability within groups and the gap between them. Host gene expression analysis results were further plotted on different groups for better understanding of the outcomes. The detailed log2Fc value along with adjusted P value is available in Supplementary file S3.

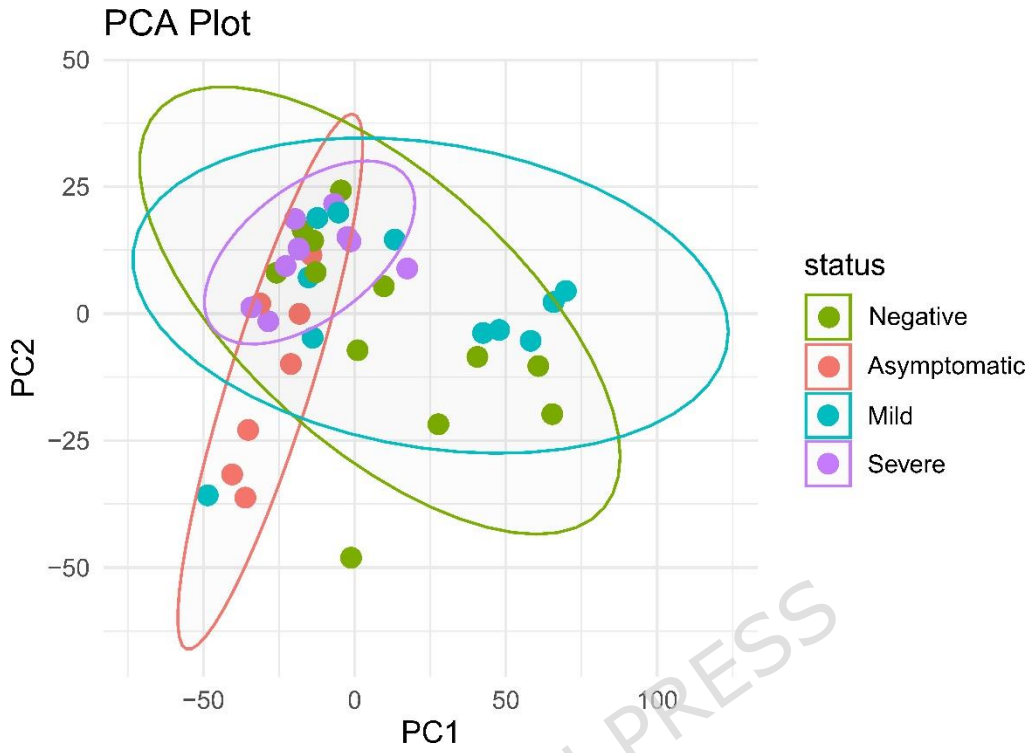

Fig 3: PCA plot showing the overlapping clusters of host gene expression data

Differential expressions of mild and negative patients showed 23 upregulated genes in the mild group than negative patients. The top five expressed genes, such as, ENSG00000229976 (lncRNA), ENSG00000087274 (*ADD1*), ENSG00000185745 (*IFIT1*), ENSG00000119922 (*IFIT2*), ENSG00000169245 (*CXCL10*) are marked in Fig 4a. After the enrichment analysis of the upregulated genes, it was evident that mostly virus associated immunological genes were upregulated in COVID-19 positive patients (Fig 4b). Cellular response to cytokine stimulus, innate immune response, response to virus, cytokine mediated signaling pathway, Type-1 interferon signaling pathway etc. were most prominent ones. This analysis followed a protein-protein interaction network analysis to ensure the association among the pathways (Fig 4c). Immune response related genes formed a cluster among themselves according to that analysis and binding regulation gene can be seen distant than the cluster.

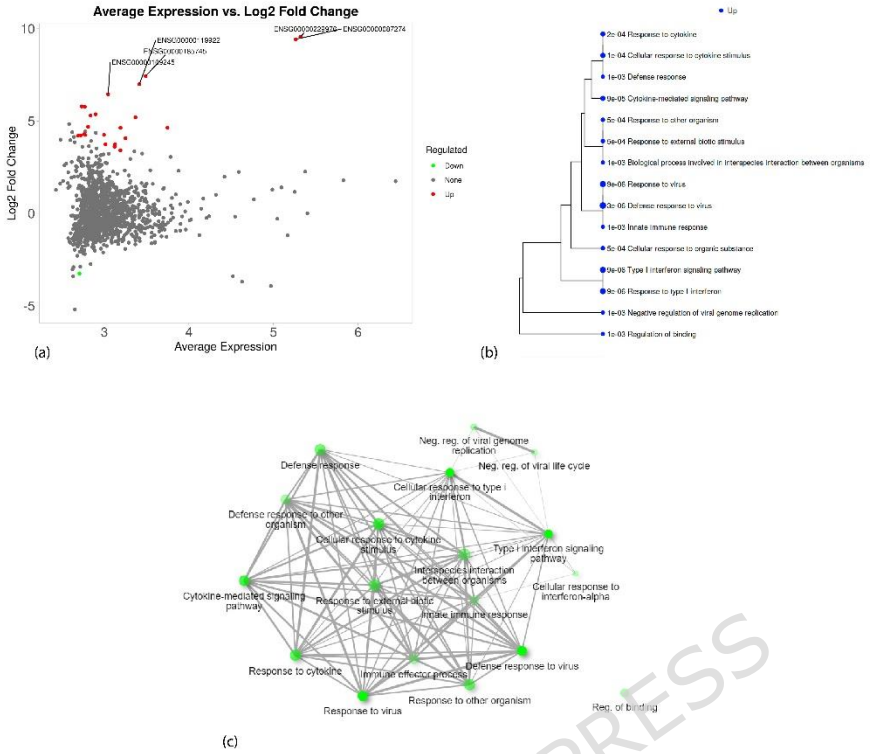

Fig 4: Differential gene expression analysis between Mild and Negative groups. Fig 4(a) shows MA plot for the differential gene expression of this group. Fig 4(b) showing enrichment analysis of the upregulated genes and Fig 4(c) showing PPI network among them.

Moreover, differential expression analysis of mild vs asymptomatic positive group and symptomatic (Mild and Severe) vs asymptomatic positive group was performed (Fig 5a, 5b). The mild and asymptomatic positive group showed 23 downregulated genes and symptomatic vs asymptomatic group showed 19 downregulated genes. The top expressed genes from both groups are shown in the MA plot. ENSG00000198522 (*GPN1*), ENSG00000160293 (*VAV2*), ENSG00000135111 (*TBX3*), ENSG00000151458 (*ANKRD50*), ENSG00000230987. Further enrichment analysis of the downregulated genes showed 16 common genes, when plotted in Venn diagram (Fig 5c). These genes showed physiological functional processes in gene ontology enrichment, and they all fall into one cluster upon network analysis among themselves (Fig 5d).

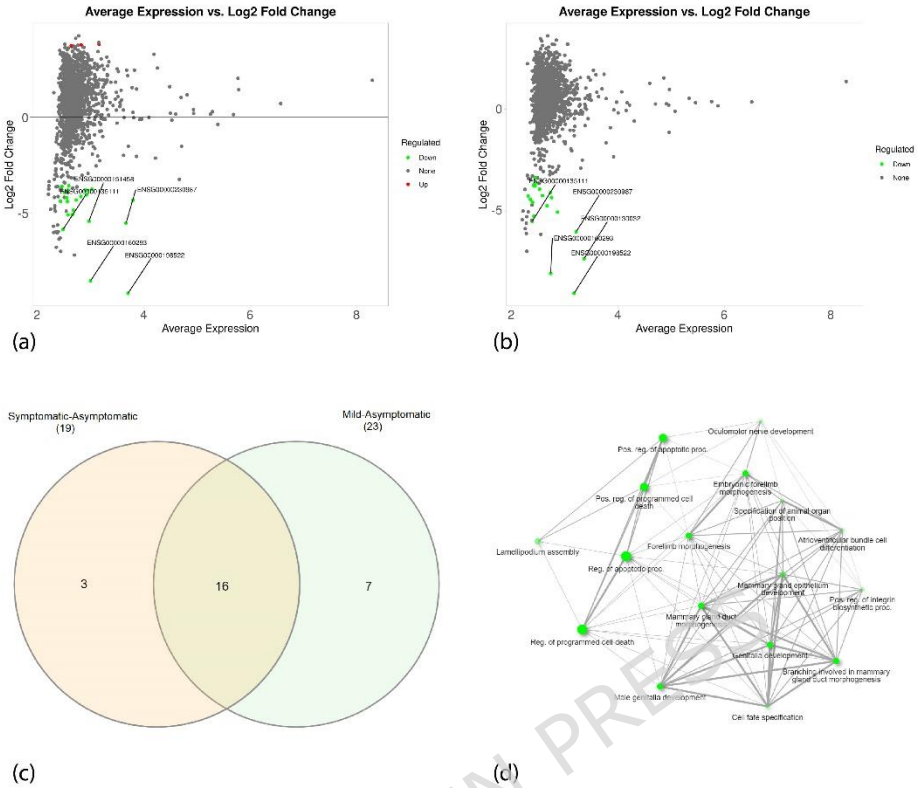

Fig 5: MA plot showing differential gene expression in two different groups. Fig 5(a) shows mild vs asymptomatic group. The top five expressed genes such as, ENSG00000198522 (*GPN1*), ENSG00000160293 (*VAV2*), ENSG00000135111 (*TBX3*), ENSG00000151458 (*ANKRD50*), ENSG00000230987 are marked here. Fig 5(b) shows symptomatic vs asymptomatic group. Top five expressed genes such as, ENSG00000198522 (*GPN1*), ENSG00000160293 (*VAV2*), ENSG00000130032 (*PRRG3*), ENSG00000230987 and ENSG00000135111 (*TBX3*) are marked here. Fig 5(c) and Fig 5(d) show the network among the common biological processes.

Furthermore, COVID-19 negative group showed 12 downregulated genes than the asymptomatic group (Fig 6a). The upregulated genes are ENSG00000210082 (*MT-RNR2*), ENSG00000211459 (*MT-RNR1*) and the top expressed downregulated genes are as follows, ENSG00000130032 (*PRRG3*), ENSG00000198522 (*GPN1*), ENSG00000087274 (*ADD1*), ENSG00000229976(lncRNA), ENSG00000287389 (lncRNA). The GO biological process enrichment analysis showed downregulation of immunological signaling genes in negative patients (Fig 6b).

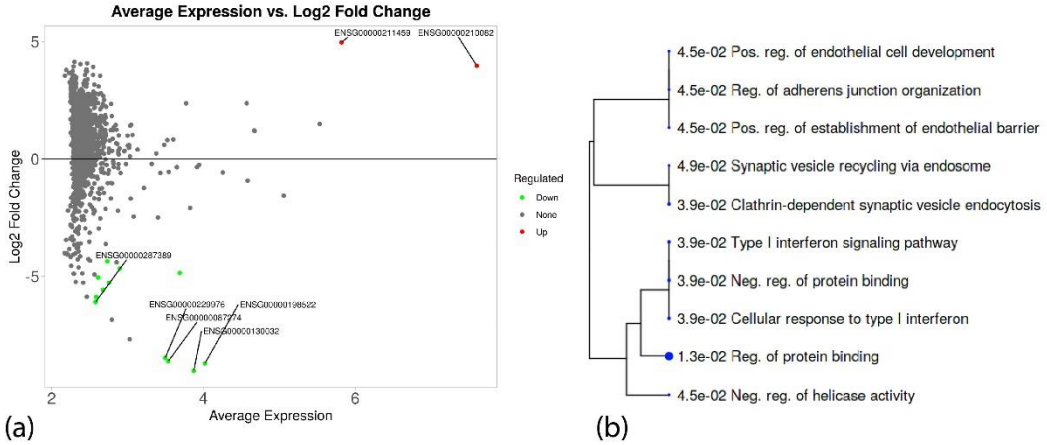

Fig 6: (a) MA plot showing differential gene expression between COVID-19 negative and asymptomatic patients. (b) showing the downregulated genes

Integrative Analysis Reveals Correlation Between Host Genes and Microbial Taxa:

The correlation analysis revealed several significant associations that highlight the direct interplay between host cellular processes and specific microbes (Fig 7; full results in Supplementary File S5). A significant positive correlation was observed between the abundance of *Pseudomonas sp. LPH1* and the expression of multiple host genes. The strongest of these was with the gene *GPN1* (Spearman's $\rho = +0.65$, adj. $p = 0.0019$). A significant positive association was also found between this microbe and *BCL2L14* (Spearman's $\rho = +0.55$, adj. $p = 0.026$).

Conversely, the analysis identified a strong and significant negative correlation between the expression of the host gene *PRRG3* and the abundance of *Moraxella osloensis* (Spearman's $\rho = -0.57$, adj. $p = 0.019$). These findings suggest that the presence of specific bacteria, such as *Pseudomonas* and *Moraxella*, may be directly linked to the modulation of host cellular pathways, providing a potential mechanistic basis for the different clinical outcomes observed in COVID-19.

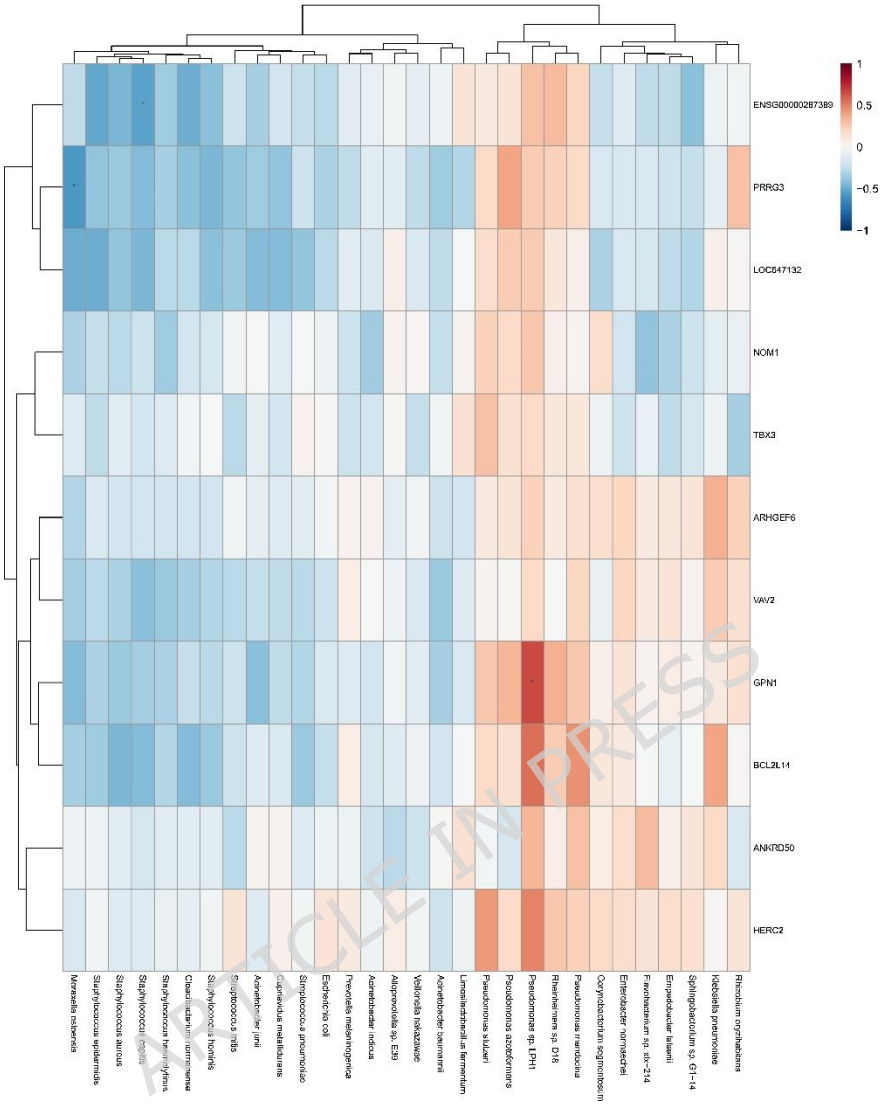

Fig 7: Correlation heatmap displaying the Spearman's correlation between top host genes (rows) and top microbial taxa (columns). Red cells indicate positive correlations, while blue cells indicate negative correlations.

COVID-19 influences the immunological signaling pathway:

KEGG pathway analysis of different cohorts showed significant results depending on severity. Further analysis on these KEGG pathways, especially immunological signaling pathways, were done to check on more detailed effects.

Pathview analysis on several immunological KEGG pathways showed significant differences among COVID-19 positive, negative, asymptomatic positive and recovered patients.

COVID-19 positive patients, irrespective of the severity, showed upregulated inflammatory response with the release of pro-inflammatory cytokines like IL-1β, IL-12 and resulted in a cytokine storm of IL-8, IL-12, MCP-1, IP-10 (Fig 8). However, when COVID-19 negative samples

were compared with asymptomatic positive samples, inflammatory cytokines were comparatively downregulated. But the cytokines were upregulated when the order was reversed i.e., asymptomatic vs negative.

An interesting pattern was found with IL-6 which is the most important cytokine for any viral infection. In COVID-19 positive cases, IL-6 was found to be moderately downregulated, which is unusual. But when the cytokine-cytokine receptor signaling was checked, IL-6 receptor was highly upregulated. Furthermore, MMP-1 was also upregulated in positive cases, which is reported to be increased with the upregulation of IL-6 [16]. As nasopharyngeal swab was used as the sample, cytokine level changes were not evident that much. If blood samples were taken, the results would be more meaningful.

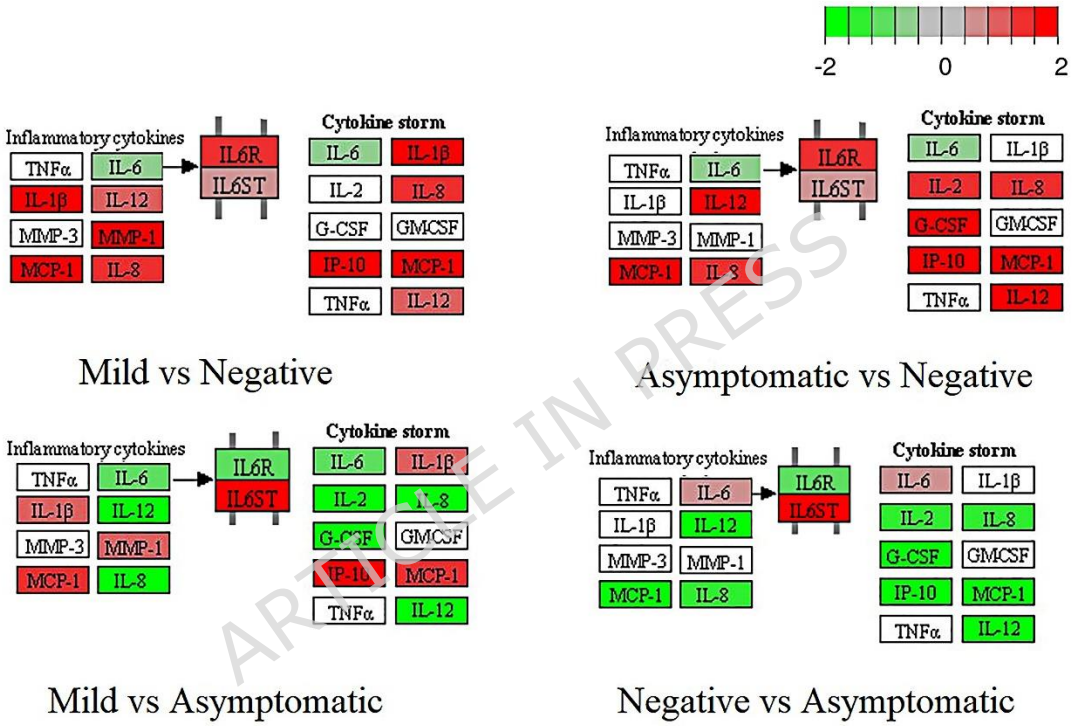

Fig 8: Changes in cytokine signaling in different study cohorts. Bright red indicates the most upregulated and bright green the most downregulated genes. KEGG [17–19] pathway maps were generated using the Pathview package in R. The panels were assembled using Microsoft Paint for layout purposes only.

Chemokines are an integral part of cytokine storm after a viral infection. Reports suggest association of CCL2, CCL3, CCL4, CCL7 and CXC subfamily with the severity of cytokine storm. These chemokines were upregulated in our COVID-19 positive cases as well (Fig 9). Asymptomatic positive patients showed a slightly different expression pattern of chemokines, especially the receptors and CXC subfamily, in comparison with COVID-19 negative patients.

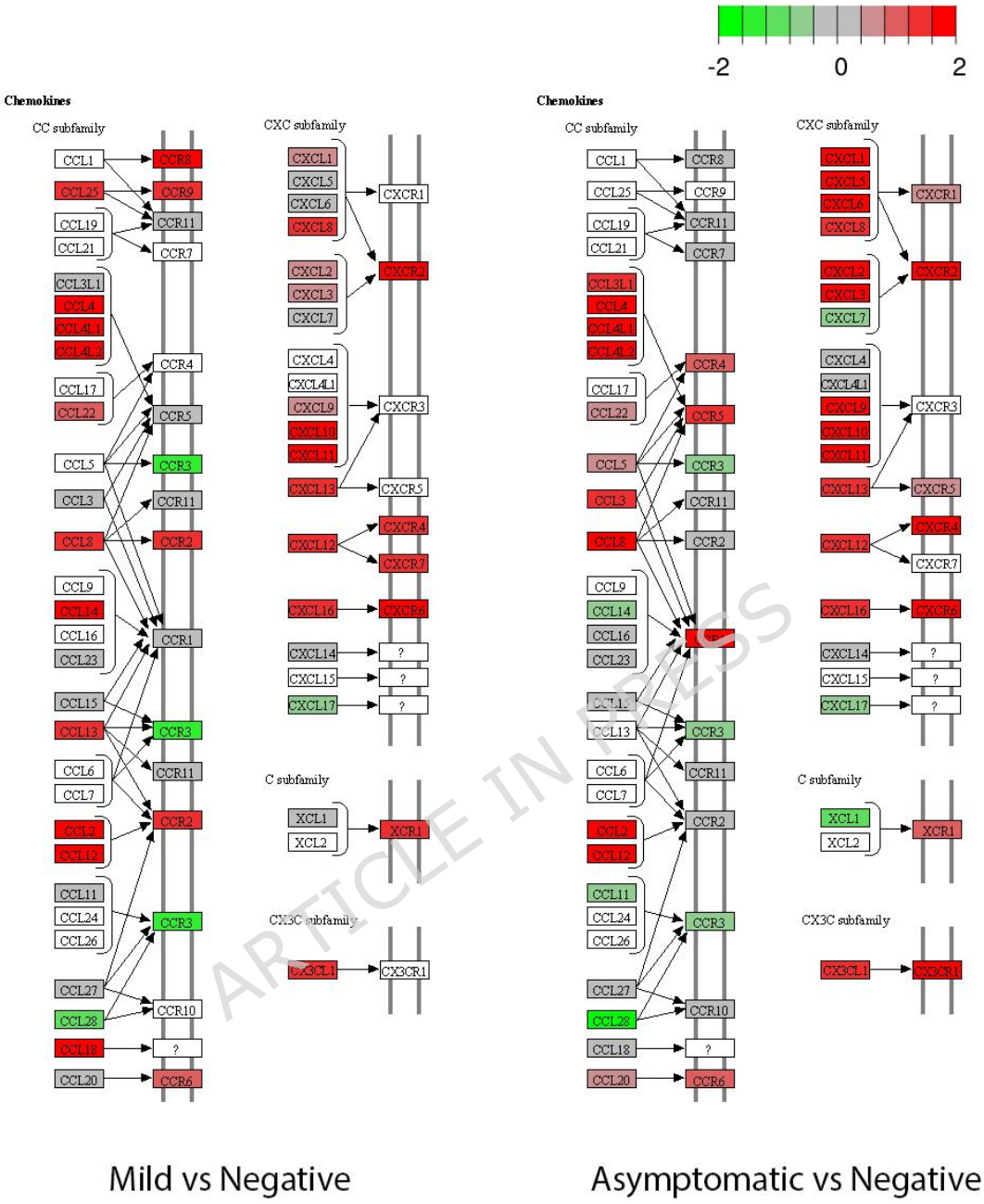

Fig 9: Changes in chemokines and their receptor signaling in different study cohorts. Here bright red indicates most upregulated, bright green, most downregulated. KEGG [17–19] pathway maps were generated using the Pathview package in R. The panels were assembled using Microsoft Paint for layout purposes only

TLRs play a major role in the initiation of innate immune responses, with the production of inflammatory cytokines, type-1 IFN and other mediators. TLR4 stimulation induced the strongest effect in terms of cytokine release (Fig 10a).

Here in this study, asymptomatic positive patients showed downregulation of TLR4 in comparison with negative samples (Fig 10b). As TLR4 plays a major role in the initiation of cytokine storm,

it may be suggested from this data that, downregulation of TLR4 could contribute to the observed asymptomatic clinical outcome.

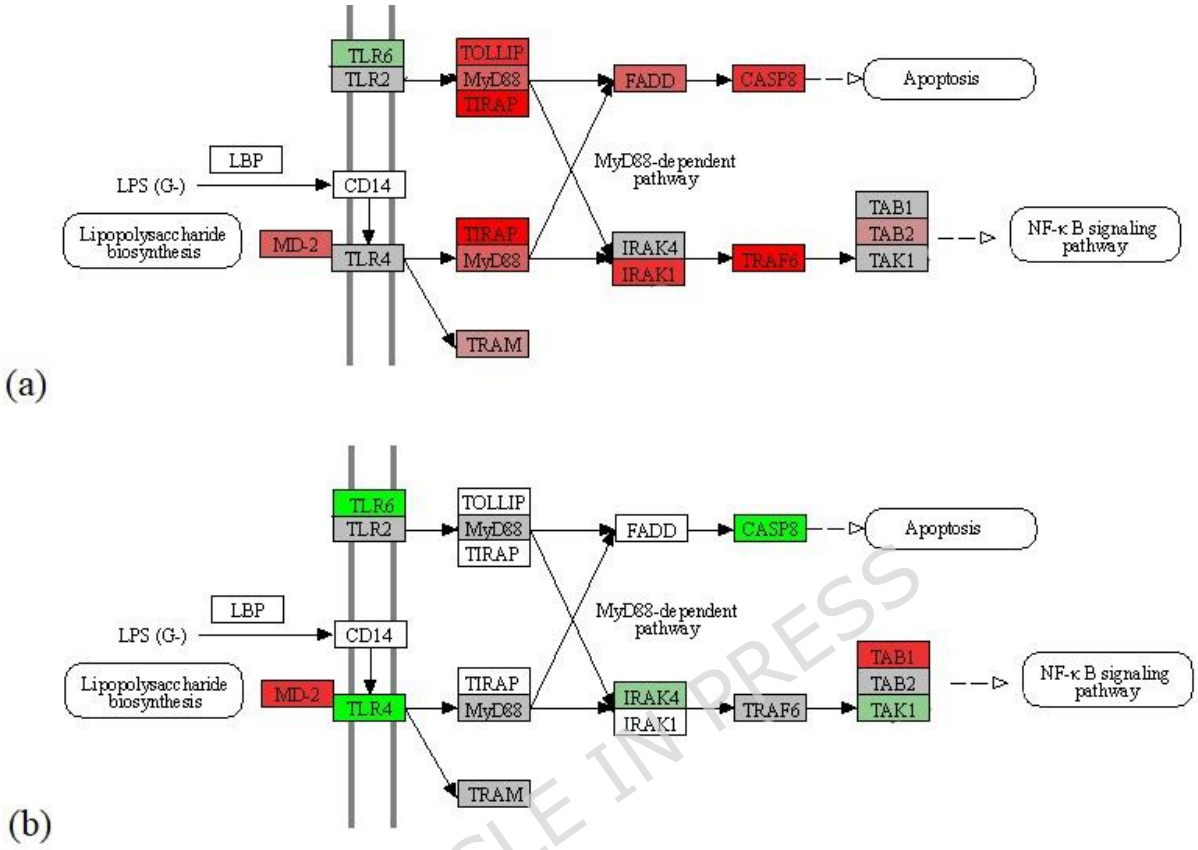

Fig 10: Partial demonstration of Toll like receptor signaling pathway in different groups. (a) Mild vs Negative and, (b) Asymptomatic Positive vs Negative. KEGG [17–19] pathway maps were generated using the Pathview package in R. The panels were assembled using Microsoft Paint for layout purposes only.

Discussion:

The interconnection between human microbiome and disease development has already been studied well by the scientific community [20–22]. Microbiome composition is shaped by various environmental factors, and in turn, influences host health and immune function. During the COVID-19 pandemic, one of the major questions arose regarding the underlying factors contributing to variable disease severity. While most of the research proved the association of comorbidity, age, sex, and immunosuppression [23–25], the potential influence of the human microbiome has also garnered attention. In particular, the microbiome's role in explaining the high prevalence of asymptomatic infections, often critical in silent transmission chains, remains an area of active investigation.

In this study, nasopharyngeal samples of different severity groups of COVID-19 patients were subjected to metatranscriptomic analysis for a better understanding of taxonomic and functional

profile. Unbiased metatranscriptomic data helps to effectively characterize the microbiome and host response in COVID-19.

Alpha diversity measured using Shannon and Simpson Diversity metrics showed significant species diversity within the sample groups in terms of prokaryotes. But widespread distribution was observed in terms of Eukaryotes (Fig 1d), suggesting greater heterogeneity among these individuals, potentially reflecting increased opportunistic fungal colonization, which has been commonly reported in severe COVID-19 cases [11]. However, between diversity analysis of different groups created a separate cluster for Asymptomatic group for both the prokaryotes and eukaryotes, highlighting the unique species diversity in the group. The rest of the groups showed a closer clustering, suggesting a more stable eukaryotic community.

The tendency to acquire more pathogenic bacteria increases with severity. From the Relative Abundance (RA) analysis, it's evident that pathogenic strains like *Acinetobacter* and *Pseudomonas* were significantly high in severe cases. Evidence showed the fungal association with the COVID-19 disease, which can sometimes affect the mortality or immune response in the host body [27,28]. In some cases, people also get fungal infection after recovering from viral diseases [29].

According to González. R et al., microbiomes have a significant and broad influence in shaping their host biology, specifically in the immunity and host defense responses [30]. It directly impacts the outcomes of infections by pathogens. This is also reflected in the microbiome metatranscriptomic analysis of different severity in this study. The asymptomatic group of patients did not show any immune response related gene expression rather than showing regular cellular function related genes. This implies a minimum amount of viral interference and an active microbial response. Moreover, the severe and mild group showed a substantial rise of immune response. The data revealed significant mechanisms pertaining to the inhibition of host type I interferon signaling and the stimulation of host autophagy, which are indicative of both viral reproduction in motion and a more regulated immune response. Additionally, there was evidence of elevated cellular stress responses and widespread viral subversion of host cellular pathways and molecular processes. Moreover, the detection of β-lactamase activity in the GO term enrichment analysis indicates not only the presence but also the functional expression of ARGs. This is further supported by the circos plot, where high-intensity signals, particularly from TEM1-D, confirm its prevalence across all severity groups, while unique ARG signatures were statistically enriched in the asymptomatic cohort. Such resistome variation may influence host-microbiome interactions, potentially modulating immune responses and contributing to reduced symptom manifestation [31–33].

However, different research works have shown how the respiratory microbiome is changing with the COVID-19 disease [34,35]. In this study, COVID-19 related differential host gene expression revealed distinct transcriptional signatures across the study cohorts. COVID-19 positive patients had twenty-three upregulated genes than the negative patients. The upregulated genes were related to responses to viral infection as cytokine storm is already reported to be associated with SARS-CoV-2 infection [36,37]. Further analysis demonstrated the highly interconnected protein-protein interaction network among the upregulated genes.

Additionally, sixteen frequently downregulated genes were identified based on the differentially expressed genes shown in the Venn diagram (Fig 5c) in order to determine the genetic signatures unique to asymptomatic positive patients. These genes were mainly linked to physiological

processes, especially those related to reproduction and development, which might be a reflection of underlying pre-existing health conditions in these people.

In addition, differential expression analysis of COVID-19 negative and asymptomatic positive group showed downregulation of immunological signaling genes in negative patients (Fig 6). As they were not affected with the SARS-CoV-2 virus infection, their viral responses were comparatively lower than the other.

The findings of this study are further strengthened by the integrated correlation analysis, which moves beyond descriptive comparisons to establish direct statistical links between host and microbial activities. For instance, the strong positive correlation between *Pseudomonas sp*. and host genes like *GPN1* and *BCL2L14* is particularly noteworthy. As *Pseudomonas* is a known opportunistic pathogen, its association with genes involved in fundamental cellular processes (GTPase activity and apoptosis regulation [38]) suggests a potential mechanism by which this microbe could influence host cell function during infection.

Similarly, the negative correlation between *Moraxella osloensis* and the host gene *PRRG3* provides a specific, testable hypothesis for future research [39]. *PRRG3* encodes a single-pass transmembrane protein containing a γ-carboxyglutamic acid (Gla) domain, consistent with a role in membrane-associated signaling or regulation [40]. The observed inverse correlation therefore represents a clear, testable link between the local microbiota and host cellular regulatory function, in line with growing evidence that microbial communities can modulate host gene expression and signaling pathways [41].

KEGG pathway analysis is widely used for understanding the biological relevance of the disease or infection with the host body [19]. Different studies have demonstrated the molecular landscape and biomarkers associated with SARS-CoV-2 viral infections using this method [42,43] but in this study a number of immunological signaling was focused on understanding the host response to the infection. Upon entry into the host and subsequent dissemination into the bloodstream, SARS-CoV-2 triggers the activation of immune cells, leading to the release of various pro-inflammatory cytokines. This, in turn, facilitates the release of inflammatory cytokines; in some cases, an exaggerated immune response leads to a cytokine storm, contributing to disease severity and multi-organ dysfunction [44]. In agreement with the available reports on “cytokine storm”, this study also found out the upregulation of some pro-inflammatory cytokines IL-1β and IL-12, which in turn upregulated cytokine storm of IL-8, IL-12, MCP-1, IP-10. In addition, asymptomatic positive samples showed upregulated cytokines against COVID-19 negative samples. The reason here lies in the threshold level of the expression [45]. As the negative samples are not affected by SARS-CoV-2, they are not expected to exhibit a cytokine storm. Though the asymptomatic positive cases may not show symptoms, they are affected by the virus. Thus, they have slight cytokine upregulation. However, this upregulation remains below the threshold needed to trigger symptoms, possibly due to internal regulatory mechanisms. Same goes for the cytokines in asymptomatic positive cases. Chemokines are reported to be involved in COVID-19 progression and severity [46] which was also seen in this study. CCL2, CCL3, CCL4, CCL7 and CXC subfamily were upregulated in the COVID-19 positive cases.

Toll-like receptors (TLRs) are vital components of the innate immune system, functioning as pattern recognition receptors that detect conserved microbial structures and initiate host defense mechanisms [47]. To date, 13 mammalian TLRs (TLR1–TLR13) have been identified, each exhibiting distinct ligand specificities and pattern recognition capabilities [48]. Though TLR4

expression is majorly dependent on lipopolysaccharide (LPS), some studies reported that it is correlated with viral disease severity [49]. This expression mediates the infection symptoms as well. Here, TLR4 has lower expression in asymptomatic positive cases than healthy individuals raising the possibility that this differential expression may contribute to the lack of symptom development in these patients. However since our study utilized bulk metatranscriptomic data from nasopharyngeal swabs, which contain a heterogeneous mixture of cell types, including epithelial cells and various immune cells [50] and TLR4 is known to be highly expressed in innate immune cells [48], it is possible that the observed downregulation in the asymptomatic cohort reflects a different cellular composition in the collected swabs (e.g., a lower proportion of immune cells) rather than a true biological downregulation within a specific cell type. We acknowledge this as a limitation of our study. Future research using single-cell sequencing would be necessary to deconvolve this effect and confirm the cell-specific expression of TLR4 [50].

In conclusion, this study reveals distinct microbiome and host transcriptomic profiles across COVID-19 severity groups, with asymptomatic cases showing unique microbial diversity, reduced immune activation, and lower TLR4 expression. Downregulation of pro-inflammatory cytokines and interleukins in this group suggests intrinsic immune regulation may limit symptom development. The detection of active antimicrobial resistance genes further underscores the need for monitoring secondary pathogens in COVID-19. These findings highlight the value of integrating microbiome and host transcriptome analyses to better understand variable disease outcomes, warranting validation in larger, diverse cohorts.

Methodology:

Ethics Statement: This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The methodology and protocol used for this study were reviewed and approved by the Human Research Ethics Review Committee, National Institute of Laboratory Medicine & Referral Center (NILMRC), Dhaka, Bangladesh. Informed verbal consent was obtained from all participants prior to sample collection, as approved by the IRB, given pandemic-related restrictions on physical contact.

Sampling and Transcriptome Sequencing: Nasopharyngeal samples from forty different people were collected for the study. COVID-19 positive and suspected individuals of different categories were included in the study after RT-PCR test. We note that patient severity classification was based on clinical symptoms and Ct values; chest X-rays or other clinical imaging were not available for assessing lung condition.

The cohort had individuals from ICU, COVID-19 positive, negative, reinfected, recovered, and asymptomatic positive patients as well. Asymptomatic positive patients were healthcare staff who had to go through routine checkup of COVID-19 RT-PCR test. However, the patients were enrolled and categorized into four cohorts based on clinical presentation, SARS-CoV-2 RT-PCR status, and duration of symptoms as described in Table 1. Detailed metadata is available in S1.

Table 1: Description of the study cohort. Here n represents the sample number

Cohort Name	Description of the Cohort
Negative Cohort (n=12)	The individuals who were negative for SARS-CoV-2 by RT-PCR and were free of symptoms of COVID-19-like illness (i.e., fever, cough, shortness of breath, loss of smell or taste) for at least two weeks.
Asymptomatic Cohort (n=7)	Asymptomatic SARS-CoV-2 positive individuals were identified through routine surveillance screening of healthcare staff. These individuals had a positive RT-PCR confirmed but were totally free of any COVID-19-like symptoms for the entire two-week period following their positive test.
Mild Cohort (n=11)	This cohort included hospitalized, RT-PCR-confirmed SARS-CoV-2 positive patients with mild to moderate symptoms of the disease. None of the patients in this cohort developed severe complications (i.e., requiring high-flow oxygen, non-invasive or invasive mechanical ventilation) over a two-week observation period following diagnosis.
Severe Cohort (n=10)	Hospitalized, RT-PCR-confirmed SARS-CoV-2 positive patients who developed severe disease requiring Intensive Care Unit (ICU) admission. Development of critical illness typically occurred between 5 to 7 days of initial symptom onset or diagnosis.

The samples were collected using universal transfer media (UTM) and transported to the laboratory under a maintained cold chain. Upon arrival, samples were immediately stored in -80°C before RNA extraction. Proper safety measures were taken to avoid RNase contamination during handling the sample. RNA was extracted using the ReliaPrep™ Viral TNA Miniprep System, Promega (USA). The RNA samples were further stored in -80°C before sequencing was performed. The RNA concentrations were measured using NanoDrop™ One (Thermo Scientific Inc. USA). Sequencing was performed at the Genomic Research Laboratory, BCSIR Dhaka Laboratory, BCSIR, Dhaka, Bangladesh. Illumina NextSeq550 platform was used for sequencing purposes, and the sequencing library was prepared using TruSeq RNA Library Preparation Kit as per the manufacturers protocol. The protocol involved rRNA removal step using Ribo-zero rRNA removal beads. Ribosomal RNA depletion efficiency was assessed computationally by quantifying reads overlapping annotated rRNA loci using BED-based genome interval intersection (bedtools). The final library concentration was quantified using a Quantus fluorometer (Promega, USA) prior to sequencing. We note that RNA integrity (e.g., RIN) was not formally assessed due to the unavailability of specialized electrophoretic equipment, and we also acknowledge that nasopharyngeal swabs frequently yield RNA of variable and often reduced integrity. However, RNA integrity and positional coverage bias were assessed computationally using RSeQC (v5.0.4) [51]. Read distribution across genomic features (CDS exons, 5′UTR, 3′UTR, and introns) was calculated using the read_distribution.py module with the GRCh38 reference annotation. This analysis was used to evaluate potential 5′–3′ degradation bias and overall transcript coverage uniformity.

Preprocessing and alignment were performed using RNA Seq Alignment app software. Quality control and adapter trimming were performed using FastQC and Trimmomatic within the pipeline,

and high-quality reads were aligned to the human reference genome (Ensembl GRCh38) using the STAR aligner [52]. Mapped reads corresponding to human transcripts were retained for host differential expression analysis. Unmapped reads were extracted and used for microbial taxonomic and functional analysis. This host read removal step ensured that microbial reads were not confounded by human sequences and reduced the likelihood of cross-alignment errors between host and microbial genomes. A list of software packages and their versions is available in the supplementary file S4.

Microbiome diversity and antibiotic resistance analysis: Taxonomic classification of the microbial reads was performed using the CZID pipeline. This pipeline determines the abundance of bacteria, archaea, viruses, and eukaryotes by aligning sequenced reads against the NCBI RefSeq database. Different microbial population (Prokaryotes and Eukaryotes) present in the nasopharyngeal samples were checked using different R packages (version 4.2), to determine the association of COVID-19 severity with microbial diversity. "Phyloseq" [53] and "Vegan" [54] were used to conduct normalization procedures via rarefaction and to assess disparities in abundances. These packages facilitated the comprehensive analysis of diversity and composition across various sample groups. Alpha diversity (differences within the sample groups) metrics were assessed employing the Wilcoxon Rank Signed test, while PERMANOVA, utilizing the Adonis test with 999 permutations (implemented through the Set.seed function), was employed to evaluate discrepancies in beta diversity. Furthermore, for the graphical representation of diversity measures and compositional analysis, R packages such as "ggpubr" and "ggplot2" were employed [55].Antibiotic resistant genes of the data were checked using CZID [56] platform, which utilizes the CARD database for resistance gene identification and classification. A Circos plot was generated using “circlize” R package [57] based on the number of hits on each resistant gene.
Sterile handling procedures were maintained throughout sample processing to reduce the risk of reagent or environmental contamination. Accordingly, interpretations of potentially pathogenic bacterial taxa and ARGs were made with caution, acknowledging the potential influence of low-level contaminant signals.
To identify differentially abundant ARGs in the asymptomatic group, raw ARG counts were analyzed using the DESeq2 package (v1.48.2) in R (v4.3.3). The analysis was performed by comparing the asymptomatic cohort against a combined symptomatic cohort (mild and severe). Genes with an FDR-adjusted p-value (padj) less than 0.05 were considered statistically significant.

Microbial metatranscriptomics, host differential gene expression and enrichment analysis: Microbial metatranscriptomics analysis was performed using Microbiome Metatranscriptomics pipeline (version 10.2) from BaseSpace, Illumina. This pipeline performs functional profiling by aligning reads against the UniRef (UniProt Reference Clusters) database for bacteria, archaea, viruses, and eukaryotes. The resulting raw data was plotted in heatmap using Pheatmap package in R. Differential gene expression of the host genome is important to determine the functional changes of human body due to diverse microbial community. DESeq2 package [58] was used for differential gene expression analysis and several other

bioinformatic tools were used for gene enrichment analysis and protein-protein interaction prediction. We used raw gene counts as the direct input for DESeq2, as recommended by the tool's authors. This method is preferred over methods using normalized counts (like TPM or RPKM) because DESeq2's statistical model inherently accounts for library size and, by performing comparisons on a per-gene basis, is not confounded by gene length differences.

Integrated Host-Microbiome Correlation Analysis: To investigate the direct relationship between the host transcriptome and the microbiome, an integrated correlation analysis was performed. First, all 11 significant differentially expressed genes (DEGs) (FDR < 0.05, |log2FC| > 1) were identified from the host transcriptome data comparing symptomatic (Mild and Severe) and asymptomatic cohorts. Separately, the top 30 most abundant microbial taxa were identified based on their mean relative abundance across the samples.

Spearman's rank correlation was then calculated for each pair of selected host genes and microbial taxa using the corr.test function from the 'psych' package in R (v4.2.0). P-values were adjusted for multiple comparisons using the False Discovery Rate (FDR) method. Correlations with an FDR-adjusted p-value < 0.05 were considered statistically significant. The results were visualized using the 'pheatmap' package.

Signaling pathway analysis: A software package Pathview [59] was used for pathway-based data integration and visualization. Pathview generates native KEGG pathway graphs [17], hence is natural and more readable for humans. Here different colors indicate the most upregulated and downregulated genes. Important immunological pathways were analyzed and compared among different study groups for finding associations using different statistical analysis.

Declaration

Ethical approval and consent to participate

The ethical permission of the protocol for sample collection from patients, sample processing, and other consecutive laboratory work was taken from the National Institute of Laboratory Medicine and Referral Center (NILMRC) of Bangladesh.

Availability of data and material

All the data related to this manuscript is available in the supplement files and all the raw data is uploaded to NCBI under the accession number of PRJNA1298058. Formal permission was taken from Kanehisa laboratories to publish the result of using KEGG software, both in print and digital, under the CC BY 4.0 open access license.

Competing interests

The authors declare that they have no competing interests

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References:

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382: 727–733. doi:10.1056/nejmoa2001017

2. Salata C, Calistri A, Parolin C, Palù G. Coronaviruses: a paradigm of new emerging zoonotic diseases. Pathog Dis. 2019;77. doi:10.1093/femspd/ftaa006

3. Tan W, Zhao X, Ma X, Wang W, Niu P, Xu W, et al. A Novel Coronavirus Genome Identified in a Cluster of Pneumonia Cases - Wuhan, China 2019-2020. China CDC Wkly. 2020;2: 61–62.

4. Du Toit A. Outbreak of a novel coronavirus. Nat Rev Microbiol. 2020;18: 123. doi:10.1038/s41579-020-0332-0

5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet Lond Engl. 2020;395: 1054–1062. doi:10.1016/S0140-6736(20)30566-3

6. Kumar A, Narayan RK, Prasoon P, Kumari C, Kaur G, Kumar S, et al. COVID-19 Mechanisms in the Human Body—What We Know So Far. Front Immunol. 2021;12. doi:10.3389/fimmu.2021.693938

7. Daamen AR, Bachali P, Owen KA, Kingsmore KM, Hubbard EL, Labonte AC, et al. Comprehensive transcriptomic analysis of COVID-19 blood, lung, and airway. Sci Rep. 2021;11. doi:10.1038/s41598-021-86002-x

8. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect. 2020;9: 761–770. doi:10.1080/22221751.2020.1747363

9. Hoque MN, Rahman MS, Ahmed R, Hossain MS, Islam MS, Islam T, et al. Diversity and genomic determinants of the microbiomes associated with COVID-19 and non-COVID respiratory diseases. Gene Rep. 2021;23: 101200. doi:10.1016/j.genrep.2021.101200

10. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet Lond Engl. 2020;395: 507–513. doi:10.1016/S0140-6736(20)30211-7

11. Hoque MN, Sarkar MMH, Rahman MS, Akter S, Banu TA, Goswami B, et al. SARS-CoV-2 infection reduces human nasopharyngeal commensal microbiome with inclusion of pathobionts. Sci Rep. 2021;11: 24042. doi:10.1038/s41598-021-03245-4

12. Avraham R, Haseley N, Fan A, Bloom-Ackermann Z, Livny J, Hung DT. A highly multiplexed and sensitive RNA-seq protocol for simultaneous analysis of host and pathogen transcriptomes. Nat Protoc. 2016;11: 1477–1491. doi:10.1038/nprot.2016.090

13. Zhang H, Ai J-W, Yang W, Zhou X, He F, Xie S, et al. Metatranscriptomic Characterization of Coronavirus Disease 2019 Identified a Host Transcriptional Classifier Associated With Immune Signaling. Clin Infect Dis. 2021;73: 376–385. doi:10.1093/cid/ciaa663

14. Haiminen N, Utro F, Seabolt E, Parida L. Functional profiling of COVID-19 respiratory tract microbiomes. Sci Rep. 2021;11. doi:10.1038/s41598-021-85750-0

15. Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, Xu N, et al. mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods. 2009;6: 377–382. doi:10.1038/nmeth.1315

16. Li Y, Samuvel DJ, Sundararaj KP, Lopes-Virella MF, Huang Y. IL-6 and high glucose synergistically upregulate MMP-1 expression by U937 mononuclear phagocytes via ERK1/2 and JNK pathways and c-Jun. J Cell Biochem. 2010;110: 248–259. doi:10.1002/jcb.22532

17. Kanehisa M, Furumichi M, Sato Y, Matsuura Y, Ishiguro-Watanabe M. KEGG: biological systems database as a model of the real world. Nucleic Acids Res. 2025;53: D672–D677. doi:10.1093/nar/gkae909

18. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci Publ Protein Soc. 2019;28: 1947–1951. doi:10.1002/pro.3715

19. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28: 27–30. doi:10.1093/nar/28.1.27

20. Waldman AJ, Balskus EP. The Human Microbiota, Infectious Disease, and Global Health: Challenges and Opportunities. ACS Infect Dis. 2018;4: 14–26. doi:10.1021/acsinfecdis.7b00232

21. Wang B, Yao M, Lv L, Ling Z, Li L. The Human Microbiota in Health and Disease. Engineering. 2017;3: 71–82. doi:10.1016/j.eng.2017.01.008

588 22. Karkman A, Lehtimäki J, Ruokolainen L. The ecology of human microbiota: dynamics and
589 diversity in health and disease. Ann N Y Acad Sci. 2017;1399: 78–92. doi:10.1111/nyas.13326

590 23. Cho SI, Yoon S, Lee H-J. Impact of comorbidity burden on mortality in patients with COVID-19
591 using the Korean health insurance database. Sci Rep. 2021;11. doi:10.1038/s41598-021-85813-2

592 24. Osibogun A, Balogun M, Abayomi A, Idris J, Kuyinu Y, Odukoya O, et al. Outcomes of COVID-19
593 patients with comorbidities in southwest Nigeria. Olusanya BO, editor. PLOS ONE. 2021;16: e0248281.
594 doi:10.1371/journal.pone.0248281

595 25. Yin T, Li Y, Ying Y, Luo Z. Prevalence of comorbidity in Chinese patients with COVID-19:
596 systematic review and meta-analysis of risk factors. BMC Infect Dis. 2021;21. doi:10.1186/s12879-021-
597 05915-0

598 26. Lopez SMC, Martin JM, Johnson M, Kurs-Lasky M, Horne WT, Marshall CW, et al. A method of
599 processing nasopharyngeal swabs to enable multiple testing. Pediatr Res. 2019;86: 651–654.
600 doi:10.1038/s41390-019-0498-1

601 27. Peng J, Wang Q, Mei H, Zheng H, Liang G, She X, et al. Fungal co-infection in COVID-19 patients:
602 evidence from a systematic review and meta-analysis. Aging. 2021;13: 7745–7757.
603 doi:10.18632/aging.202742

604 28. Verweij PE, Gangneux J-P, Bassetti M, Brüggemann RJM, Cornely OA, Koehler P, et al. Diagnosing
605 COVID-19-associated pulmonary aspergillosis. Lancet Microbe. 2020;1: e53–e55. doi:10.1016/S2666-
606 5247(20)30027-6

607 29. Canning B, Senanayake V, Burns D, Moran E, Dedicoat M. Post-influenza aspergillus ventriculitis.
608 Clin Infect Pract. 2020;7–8: 100026. doi:10.1016/j.clinpr.2020.100026

609 30. González R, Elena SF. The Interplay between the Host Microbiome and Pathogenic Viral
610 Infections. Prasad VR, editor. mBio. 2021;12. doi:10.1128/mbio.02496-21

611 31. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, et al. Recovery of gut
612 microbiota of healthy adults following antibiotic exposure. Nat Microbiol. 2018;3: 1255–1265.
613 doi:10.1038/s41564-018-0257-9

614 32. Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, et al. Alterations in Gut Microbiota of Patients
615 With COVID-19 During Time of Hospitalization. Gastroenterology. 2020;159: 944-955.e8.
616 doi:10.1053/j.gastro.2020.05.048

617 33. Umeda L, Torres A, Kunihiro BP, Rubas NC, Wells RK, Phankitnirundorn K, et al. Immuno-
618 Microbial Signature of Vaccine-Induced Immunity against SARS-CoV-2. Vaccines. 2024;12: 637.
619 doi:10.3390/vaccines12060637

620 34. Ng DL, Granados AC, Santos YA, Servellita V, Goldgof GM, Meydan C, et al. A diagnostic host
621 response biosignature for COVID-19 from RNA profiling of nasal swabs and blood. Sci Adv. 2021;7:
622 eabe5984. doi:10.1126/sciadv.abe5984

623 35. Hyblova M, Hadzega D, Babisova K, Krumpolec P, Gnip A, Sabaka P, et al. Metatranscriptome
624 Analysis of Nasopharyngeal Swabs across the Varying Severity of COVID-19 Disease Demonstrated

Unprecedented Species Diversity. Microorganisms. 2023;11: 1804. doi:10.3390/microorganisms11071804

36. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395: 497–506. doi:10.1016/s0140-6736(20)30183-5

37. Liu Y, Zhang C, Huang F, Yang Y, Wang F, Yuan J, et al. Elevated plasma levels of selective cytokines in COVID-19 patients reflect viral load and lung injury. Natl Sci Rev. 2020;7: 1003–1011. doi:10.1093/nsr/nwaa037

38. Nuñez G, Clarke MF. The Bcl-2 family of proteins: regulators of cell death and survival. Trends Cell Biol. 1994;4: 399–403. doi:10.1016/0962-8924(94)90053-1

39. Lin Z, Jiang Y, Liu H, Yang J, Yang B, Zhang K, et al. Airway microbiota and immunity associated with chronic obstructive pulmonary disease severity. J Transl Med. 2025;23: 962. doi:10.1186/s12967-025-06986-2

40. Zhang E, He J, Zhang H, Shan L, Wu H, Zhang M, et al. Immune-Related Gene-Based Novel Subtypes to Establish a Model Predicting the Risk of Prostate Cancer. Front Genet. 2020;11: 595657. doi:10.3389/fgene.2020.595657

41. Daniel N, Lécuyer E, Chassaing B. Host/microbiota interactions in health and diseases—Time for mucosal microbiology! Mucosal Immunol. 2021;14: 1006–1016. doi:10.1038/s41385-021-00383-w

42. Tian W, Zhang N, Jin R, Feng Y, Wang S, Gao S, et al. Immune suppression in the early stage of COVID-19 disease. Nat Commun. 2020;11. doi:10.1038/s41467-020-19706-9

43. Fulzele S, Sahay B, Yusufu I, Lee TJ, Sharma A, Kolhe R, et al. COVID-19 Virulence in Aged Patients Might Be Impacted by the Host Cellular MicroRNAs Abundance/Profile. Aging Dis. 2020;11: 509–522. doi:10.14336/AD.2020.0428

44. Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. Front Immunol. 2020;11: 1708. doi:10.3389/fimmu.2020.01708

45. Tjan LH, Furukawa K, Nagano T, Kiriu T, Nishimura M, Arii J, et al. Early Differences in Cytokine Production by Severity of Coronavirus Disease 2019. J Infect Dis. 2021;223: 1145–1149. doi:10.1093/infdis/jiab005

46. Coperchini F, Chiovato L, Ricci G, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: Further advances in our understanding the role of specific chemokines involved. Cytokine Growth Factor Rev. 2021;58: 82–91. doi:10.1016/j.cytogfr.2020.12.005

47. Cui J, Chen Y, Wang HY, Wang R-F. Mechanisms and pathways of innate immune activation and regulation in health and cancer. Hum Vaccines Immunother. 2014;10: 3270–3285. doi:10.4161/21645515.2014.979640

48. Takeda K, Kaisho T, Akira S. Toll-Like Receptors. Annu Rev Immunol. 2003;21: 335–376. doi:10.1146/annurev.immunol.21.120601.141126

661 49. Sahanic S, Hilbe R, Dünser C, Tymoszuk P, Löffler-Ragg J, Rieder D, et al. SARS-CoV-2 activates
662 the TLR4/MyD88 pathway in human macrophages: A possible correlation with strong pro-inflammatory
663 responses in severe COVID-19. Heliyon. 2023;9: e21893. doi:10.1016/j.heliyon.2023.e21893

664 50. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, et al. SARS-CoV-2 Receptor
665 ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell
666 Subsets across Tissues. Cell. 2020;181: 1016-1035.e19. doi:10.1016/j.cell.2020.04.035

667 51. Wang L, Wang S, Li W. RSeQC: quality control of RNA-seq experiments. Bioinforma Oxf Engl.
668 2012;28: 2184–2185. doi:10.1093/bioinformatics/bts356

669 52. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-
670 seq aligner. Bioinforma Oxf Engl. 2013;29: 15–21. doi:10.1093/bioinformatics/bts635

671 53. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and
672 Graphics of Microbiome Census Data. Watson M, editor. PLoS ONE. 2013;8: e61217.
673 doi:10.1371/journal.pone.0061217

674 54. Oksane J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, et al. The vegan package.
675 Community ecology package. 2007;10: 631–637.

676 55. Kassambara A. ggpubr: "ggplot2" Based Publication Ready Plots. 2025. Available:
677 https://rpkgs.datanovia.com/ggpubr/

678 56. Kalantar KL, Carvalho T, De Bourcy CFA, Dimitrov B, Dingle G, Egger R, et al. IDseq—An open
679 source cloud-based pipeline and analysis service for metagenomic pathogen detection and monitoring.
680 GigaScience. 2020;9. doi:10.1093/gigascience/giaa111

681 57. Gu Z, Gu L, Eils R, Schlesner M, Brors B. circlize Implements and enhances circular visualization in
682 R. Bioinforma Oxf Engl. 2014;30: 2811–2812. doi:10.1093/bioinformatics/btu393

683 58. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq
684 data with DESeq2. Genome Biol. 2014;15: 550. doi:10.1186/s13059-014-0550-8

685 59. Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration
686 and visualization. Bioinforma Oxf Engl. 2013;29: 1830–1831. doi:10.1093/bioinformatics/btt285

687

688