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COVID-19 como Evento Disruptivo na Ecologia Viral: Repercussões para as Síndromes Respiratórias Agudas Graves e para a Interpretação das Sequelas Pós-Infecciosas

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Tese de Doutorado apresentada ao Curso de Pós- Graduação em Ciências Biológicas – área de concentração Imunologia e Doenças Infecto- Parasitárias como pré requisito para obtenção do Título de Doutora em Ciências Biológicas.

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"Se vi mais longe, foi por estar sobre ombros de gigantes." (*Isaac Newton*)

RESUMO

A pandemia de COVID-19 atuou como um evento disruptivo na ecologia dos vírus respiratórios, alterando padrões de sazonalidade, cocirculação e interpretação clínica das síndromes respiratórias agudas e de suas sequelas pós-infecciosas. Esta tese integra evidências de três abordagens complementares que cobrem diferentes fases e consequências da pandemia em Minas Gerais, Brasil. No período pandêmico (fevereiro a setembro de 2021), foi conduzido um estudo retrospectivo na Zona da Mata Mineira com amostras respiratórias encaminhadas ao serviço de diagnóstico molecular do ICB/UFJF, combinando vigilância molecular por sequenciamento de genoma completo para caracterização de variantes do SARS-CoV-2 e triagem de outros vírus respiratórios (HRV, RSV e Influenza A/B), além de análises clínico-epidemiológicas e modelagem estatística. Nesse contexto, observou-se predominância da variante Gama, associada a maior gravidade clínica quando comparada à Delta (OR ~3,0; IC95%: 1,21–7,32), e evidências de redução da circulação de Influenza e RSV durante momentos de maior intensidade da COVID-19. Adicionalmente, comorbidades como diabetes (OR 2,14; IC95%: 1,21–3,79) e obesidade (OR 7,02; IC95%: 1,49–33,00) foram associadas à infecção por SARS-CoV-2, e doença cardíaca mostrou associação independente com infecção por Gama (aOR 3,54; IC95%: 1,99–6,29). No cenário pós-pandêmico (2023–2024), um segundo estudo retrospectivo avaliou casos de síndrome respiratória aguda grave (SRAG) das regionais de Barbacena e Leopoldina, processados pelo Centro Colaborador da SES/MG no ICB/UFJF, com aplicação de RT-qPCR (incluindo ensaio multiplex) e integração a dados clínico-epidemiológicos. Foram analisadas 264 amostras de 36 municípios, com predominância de residentes em área urbana (90,77%). Observou-se um perfil etário heterogêneo (mediana de 14 anos) e detecção de RSV (14,77%), HRV (11,36%), Influenza A/B (9,46%) e SARS-CoV-2 (7,95%), com coinfecções pouco frequentes (2,65%). Destacou-se a elevada proporção de SRAG sem etiologia definida (53,78%), indicando lacunas diagnósticas persistentes apesar da ampliação da capacidade de testagem molecular. O padrão temporal foi atípico para Influenza (com pico em agosto de 2024) e revelou alta carga de hospitalizações associadas ao RSV (com aumento marcado entre março e junho de 2024 e pico em maio), enquanto o SARS-CoV-2 apresentou circulação mais esporádica. Em ambos os períodos, análises multivariadas reforçam que a sobreposição clínica entre diferentes vírus permanece um obstáculo central ao manejo sindrômico, e que coinfecções não se associaram de forma consistente a piores desfechos nos cenários estudados. Por fim, uma síntese narrativa discute a COVID longa como condição pós-infecciosa multissistêmica, com definições heterogêneas, ausência de biomarcadores e impacto relevante sobre qualidade de vida e sistemas de saúde, agravada por desinformação, estigma e desigualdades de acesso ao cuidado. Em conjunto, os achados reforçam a necessidade de vigilância integrada e sustentada de vírus respiratórios para além do SARS-CoV-2, expansão do acesso a painéis diagnósticos moleculares multiplex, fortalecimento de estratégias de vacinação e integração entre laboratório, clínica e saúde pública para reduzir morbimortalidade e aumentar a preparação frente a futuras emergências respiratórias.

Palavras-chave: vírus respiratórios; vigilância molecular; SARS-CoV-2; SRAG; sazonalidade; COVID longa.

ABSTRACT

The COVID-19 pandemic acted as a disruptive event in respiratory viral ecology, reshaping seasonality, co-circulation patterns, and the clinical interpretation of acute respiratory syndromes and their post-infectious sequelae. This thesis integrates evidence from three complementary approaches addressing distinct phases and consequences of the pandemic in Minas Gerais, Brazil. During the pandemic phase (February to September 2021), a retrospective study in the Zona da Mata region analyzed respiratory samples processed by the molecular diagnostics service at ICB/UFJF, combining genomic surveillance (whole-genome sequencing) to characterize SARS-CoV-2 variants with molecular screening for other major respiratory viruses (human rhinovirus [HRV], respiratory syncytial virus [RSV], and influenza A/B), alongside clinical–epidemiological analyses and statistical modeling. In this period, the Gamma variant predominated and was associated with greater clinical severity compared with Delta (OR ~3.0; 95% CI: 1.21–7.32), with evidence of reduced influenza and RSV circulation during peaks of COVID-19 activity. Comorbidities such as diabetes (OR 2.14; 95% CI: 1.21–3.79) and obesity (OR 7.02; 95% CI: 1.49–33.00) were associated with SARS-CoV-2 infection, and heart disease showed an independent association with Gamma infection (aOR 3.54; 95% CI: 1.99–6.29). In the post-pandemic period (2023–2024), a second retrospective study evaluated severe acute respiratory illness cases (SRAG/SARI) from the Barbacena and Leopoldina regions processed at the SES/MG collaborating center at ICB/UFJF, integrating RT-qPCR (including multiplex assays) with clinical and epidemiological data. Among 264 samples from 36 municipalities, most cases were from urban residents (90.77%), with a heterogeneous age distribution (median 14 years). RSV (14.77%), HRV (11.36%), influenza A/B (9.46%), and SARS-CoV-2 (7.95%) were detected, while coinfections were uncommon (2.65%). A high proportion of SRAG/SARI cases remained without an identified viral etiology (53.78%), underscoring persistent diagnostic gaps despite expanded molecular testing capacity. Temporal patterns were atypical for influenza (peak in August 2024) and indicated a substantial RSV-related hospitalization burden (increase from March to June 2024, peaking in May), whereas SARS-CoV-2 circulation was more sporadic. Across both phases, multivariate analyses support that clinical overlap among respiratory viruses remains a major barrier to syndromic management, and viral coinfections were not consistently associated with worse outcomes in the studied settings. Finally, a narrative synthesis discusses long COVID as a multisystem post-infectious condition characterized by heterogeneous definitions, lack of biomarkers, and significant impacts on quality of life and healthcare systems, exacerbated by misinformation, stigma, and inequalities in access to care. Overall, the findings reinforce the need for sustained, integrated respiratory virus surveillance beyond SARS-CoV-2, broader access to multiplex molecular diagnostics, strengthened vaccination strategies, and coordinated public health approaches that connect laboratory evidence, clinical decision-making, and preparedness for future respiratory health emergencies.

Keywords: respiratory viruses; molecular surveillance; SARS-CoV-2; SARI/SRAG; seasonality; long COVID.

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CAPÍTULO 1

LISTA DE ABREVIATURAS E SIGLAS

ACE2	Enzima conversora de angiotensina 2
ANOVA	Teste estátistico de análise de variância
ARIs	Infecções respiratórias agudas
BBB	barreira hematoencefálica
CEMIC	Centro de Estudos em Microbiologia
CIs	Intervalos de Confiança
COVID-19	Infecção respiratória aguda causada pelo coronavírus SARS-CoV-2.
Ct	Limiar de ciclo
CT scan	Tomografia computadorizada
DNA	Acido Desoxirribonucleico
EBV	Vírus Epstein-Barr
FASTA	Formato de arquivo de texto que armazena sequências biológicas (DNA, RNA ou proteínas)
FLU	Influenza vírus
GISRS	*Global Influenza Surveillance and Response System*
GRS	Gerências Regionais de Saúde
HAdV	Vírus adenovírus
HCoVs	coronavírus humanos sazonais
hMPV	metapneumovírus humano
HPIV	Vírus parainfluenza
HRQoL	Escala de qualidade de vida relacionada à Saúde
HRV	Vírus rinovírus
ICU	Unidade de terapia intensiva
IRAS	Infecções respiratórias agudas
ITpS	Instituto Todos pela Saúde
LCA	Análise de Classes latentes
LD	Dicroísmo Linear
ME/CFS	Encefalomielite miálgica/síndrome da fadiga crônica
NPIs	Intervenções não farmacológicas
PASC	Sequelas pós fase aguda da COVID-19
PCA	Análise de componente principal

PHCUs	Unidades de atenção primária
POTS	síndrome de taquicardia postural ortostática
RNA	Ácido Ribonucleico
RSV	Vírus sincicial respiratório
RT-qPCR	Transcriptase Reversa, PCR quantitativa em Tempo Real
SARI	Infecção respiratoria aguda grave
SARS-CoV-2	Vírus corona vírus 2 da síndrome respiratoria aguda grave
SC2	Vírus corona vírus 2 da síndrome respiratoria aguda grave
SD	Desvio padrão
SES/MG	Secretaria de Estado de Saúde de Minas Gerais
SpO_2	saturação periférica de oxigênio
SPSS v20	*Software* par análise de dados
SYBR	corante fluorescente de cianina usado em biologia molecular
VOCs	Variantes genéticas de preocupação
WHO	Organização Mundial da Saúde

SUMÁRIO

1 INTRODUÇÃO

As infecções respiratórias agudas (IRAs) representam uma das principais causas de morbidade e mortalidade em escala global, impactando indivíduos de todas as idades, mas com maior gravidade entre crianças, idosos e pessoas com comorbidades crônicas (Soares et al., 2025; Chow et al., 2022.).. Os vírus respiratórios estão entre os agentes mais frequentes associados a essas condições, mas a sobreposição clínica dos sintomas dificulta a diferenciação entre os diversos patógenos, tornando imprescindível o diagnóstico laboratorial para orientar condutas terapêuticas e estratégias de controle epidemiológico. (Chow et al., 2022; Sapra et al., 2022).

Diversos vírus estão implicados na etiologia das IRAs, incluindo vírus sincicial respiratório (RSV), influenza A e B, rinovírus (HRV), vírus parainfluenza (HPIV), adenovírus (HAdV) e coronavírus, com destaque recente para o SARS-CoV-2 (Papagiannis et al., 2023; Soares et al., 2025; Sapra et al., 2022). Em populações pediátricas, o RSV é frequentemente identificado como principal agente (Papagiannis et al., 2023).

Antes da pandemia de COVID-19, esses vírus exibiam padrões epidemiológicos bem caracterizados. Vírus como influenza e RSV eram conhecidos por seus picos sazonais de inverno, enquanto outros, como o rinovírus, estavam presentes o ano todo, contribuindo para uma carga constante de doenças respiratórias em todo o mundo (Costanza et al., 2022; Watanabe et al., 2011).

Com a disseminação global do SARS-CoV-2, esses padrões foram profundamente alterados. Medidas não farmacológicas como o uso de máscaras, distanciamento social e restrição de mobilidade impactaram diretamente a circulação de diversos vírus respiratórios (You et al., 2025; Chow et al., 2022). A partir de 2020, no entanto, o predomínio quase exclusivo do SARS-CoV-2 instaurou uma nova dinâmica viral, exigindo reestruturação da vigilância epidemiológica com foco ampliado (Kampenusa et al., 2024).

Nesse contexto, o RT-qPCR multiplex em tempo real consolidou-se como ferramenta diagnóstica essencial, permitindo a detecção simultânea de múltiplos vírus respiratórios em uma única amostra clínica (Kampenusa et al., 2024; Trifonova et al. 2024). Essa abordagem tem sido fundamental para identificar casos de codetecção e coinfecção viral, fenômenos que se tornaram mais evidentes com o retorno gradual da circulação de vírus anteriormente

suprimidos durante a pandemia (Kampenusa et al., 2024; Trifonova et al. 2024; Martinón-Torres et al. 2024).

Estudos conduzidos em diferentes países demonstraram que vírus como HRV, adenovírus e enterovírus são frequentemente envolvidos em coinfecções, mesmo em painéis diagnósticos originalmente voltados para SARS-CoV-2, influenza e RSV (Krashias et al., 2025; Kampenusa et al., 2024). No entanto, a vigilância laboratorial enfrenta limitações importantes, sobretudo pela utilização seletiva de painéis que restringem a testagem a populações específicas, como pacientes imunocomprometidos, hospitalizados ou pediátricos (Mostafa et al., 2024).

Essa seletividade gera distorções nos dados e pode subestimar a real prevalência de vírus relevantes. Um exemplo é o do enterovírus/rinovírus, cuja alta taxa de positividade foi observada apenas em locais com escopo ampliado de testagem. A ausência de uma abordagem diagnóstica abrangente limita a sensibilidade da vigilância e revela lacunas críticas nos sistemas de informação em saúde pública (Mostafa et al., 2024).

Com a redução da intensidade das ondas pandêmicas e a ampliação da cobertura vacinal contra a COVID-19, observou-se a transição para um novo equilíbrio epidemiológico. (Sberna et al., 2025) A queda progressiva na prevalência do SARS-CoV-2 permitiu o ressurgimento de vírus sazonais como influenza e RSV, especialmente entre crianças pequenas e idosos, enquanto o HRV manteve sua circulação contínua. Em contrapartida, o SARS-CoV-2 passou a apresentar comportamento mais errático e imprevisível (Sberna et al., 2025; Kampenusa et al., 2024; Hanage et al., 2024)

Essas alterações podem ser parcialmente explicadas pelo fenômeno da interferência viral, em que a presença de um vírus interfere na replicação ou na propagação de outro, por meio de mecanismos como a indução de interferons tipo I ou competição por células hospedeiras. Entender essas interações é fundamental para prever padrões sazonais e orientar estratégias de imunização (Sberna et al., 2025; Watanabe et al., 2011).

Dessa forma, o cenário atual das IRAs é marcado por uma recomposição dinâmica das interações virais e pela coexistência de surtos de diferentes agentes. Tal complexidade reforça a necessidade de sistemas de vigilância ampliados, sensíveis e continuamente adaptáveis às novas realidades epidemiológicas (Sberna et al., 2025).

A pandemia de COVID-19, provocou uma das maiores crises sanitárias da história recente. A rápida propagação do vírus, favorecida por sua alta transmissibilidade e potencial para infecções assintomáticas, evidenciou as fragilidades dos sistemas de saúde globais e demandou medidas de contenção sem precedentes (Manirambona et al., 2025; Krashias et al., 2025). A ausência de imunidade populacional e as características virológicas do patógeno resultaram em impactos sanitários, sociais e econômicos de grande escala (Bambra et al., 2020; Castro et al., 2021).

Frente a esse cenário, a vigilância genômica tornou-se peça-chave para monitorar a evolução viral e adaptar as respostas de saúde pública. Variantes como Alpha, Delta e Ômicron foram identificadas e caracterizadas por sua transmissibilidade, escape imunológico e impacto clínico, exigindo ajustes nas estratégias vacinais e no manejo clínico (Croda et al., 2020; Faria et al., 2021; Viana et al., 2022). O sequenciamento genético contribuiu decisivamente para rastrear cadeias de transmissão, prever novos surtos e apoiar o desenvolvimento de vacinas atualizadas (Mojica-Crespo et al., 2020).

Além das manifestações agudas, a infecção por SARS-CoV-2 revelou um espectro persistente de sintomas, desafiando o paradigma das IRAs como doenças autolimitadas. Surgiu, então, a condição conhecida como COVID longa, definida pela persistência de sintomas como fadiga, dispneia, cefaleia, distúrbios de memória e alterações neuropsiquiátricas por semanas ou meses após a infecção aguda (Brasil, 2022).

A fisiopatologia da COVID longa ainda é objeto de intensa investigação. Fatores como a resposta imunológica desregulada, características virais específicas (incluindo variantes), comorbidades prévias e até a composição da microbiota respiratória superior têm sido implicados na manutenção dos sintomas (Ma et al., 2021; Boufidou et al., 2023; Byambassuen et al., 2024). Estudos multicêntricos vêm integrando dados clínicos, genômicos e epidemiológicos para melhor caracterizar os pacientes afetados e propor intervenções eficazes (Marra et al., 2023; Davis et al., 2023).

A análise de amostras coletadas no início da pandemia, em uma fase marcada pela ausência de imunidade vacinal, oferece uma oportunidade única para estudar a relação entre variantes virais e desfechos clínicos. Esses dados podem contribuir significativamente para o desenvolvimento de protocolos de reabilitação e vigilância pós-aguda (Xiao et al., 2024).

Embora diversos estudos tenham caracterizado a circulação de vírus respiratórios em momentos isolados da pandemia, ainda são escassos os trabalhos que integram análises clínicas, epidemiológicas e laboratoriais ao longo de todo o curso da pandemia. Esta tese visa preencher essa lacuna por meio da articulação de três estudos complementares que abrangem diferentes fases da crise sanitária: (1) a vigilância integrada de vírus respiratórios durante a pandemia; (2) a reorganização da circulação viral no período pós-pandêmico; e (3) a caracterização clínica de casos de COVID longa.

Ao integrar essas abordagens, o trabalho contribui para fortalecer a vigilância epidemiológica, melhorar o diagnóstico diferencial de infecções respiratórias e aprofundar a compreensão das repercussões agudas e crônicas da infecção por SARS-CoV-2.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Integrar achados clínicos, epidemiológicos e laboratoriais sobre infecções respiratórias virais em diferentes momentos da pandemia de COVID-19, com ênfase nos desfechos agudos e crônicos.

2.2 OBJETIVOS ESPECÍFICOS

I. Descrever o perfil clínico-epidemiológico de vírus respiratórios durante a pandemia;
II. Analisar a circulação e os sintomas associados a vírus respiratórios no período pós- pandêmico;
III. Discutir as implicações clínicas e sociais da COVID longa como sequela da infecção por SARS-CoV-2.

CAPÍTULO 1:

EPIDEMIOLOGICAL AND CLINICAL PROFILE OF RESPIRATORY VIRAL INFECTIONS IN 2021: INTEGRATED SURVEILLANCE DURING THE COVID- 19 PANDEMIC

Taylor & Francis
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Future Virology

Epidemiological and Clinical Profile of Respiratory Viral Infections in 2021: Integrated Surveillance During the COVID-19 Pandemic

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Title: Epidemiological and Clinical Profile of Respiratory Viral Infections in 2021: Integrated Surveillance During the COVID-19 Pandemic

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Abstract

Background: The COVID-19 pandemic disrupted the seasonality and detection patterns of other respiratory viruses, complicating syndromic surveillance and clinical management in several regions, including Brazil. Objectives: To describe the epidemiological, clinical and hospitalization profiles of patients with laboratory-confirmed respiratory viral infections diagnosed in 2021, comparing SARS-CoV-2 variants, influenza B, respiratory syncytial virus (RSV), human rhinovirus (HRV) and negative cases. Methods: We conducted a retrospective analysis of molecular diagnostic results and clinical–epidemiological data from 337 patients/samples. Analyses included logistic regression to estimate odds ratios (ORs) for risk factors and principal component analysis (PCA) to explore symptom clustering. Findings: SARS-CoV-2 (Gamma variant), HRV, RSV and influenza B were the most frequently detected viruses. PCA revealed virus-specific symptom clusters, with notable overlap among pathogens. Compared with Delta, Gamma showed higher clinical severity (OR~3.0; CI95%: 1.21–7.32). Diabetes and obesity were significantly associated with COVID-19. Coinfections were infrequent and not linked to greater severity. Main conclusions: Findings support integrated respiratory surveillance using multiplex PCR in sentinel settings, variant-aware clinical screening and timely genomic characterization to guide care pathways and inform local public health actions.

Keywords: Respiratory Tract Infections, Public Health Surveillance, SARS-CoV-2, COVID-19 Pandemic

1. Introduction

Respiratory viruses are among the leading causes of acute respiratory infections (ARIs) worldwide, contributing substantially to morbidity, hospitalizations and mortality, especially among young children, older adults and individuals with underlying health conditions [1,2]. The seasonal circulation of pathogens such as influenza, respiratory syncytial virus (RSV) and human rhinovirus (HRV) traditionally follows predictable patterns in most regions, facilitating public health planning and healthcare resource allocation [3-5].

However, the onset of the COVID-19 pandemic in early 2020 profoundly disrupted these patterns globally. Non-pharmaceutical interventions (NPIs), including social distancing, mask use, school closures and travel restrictions, were effective not only in curbing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission but also led to in producing unprecedented declines in the detection of other respiratory viruses [6,7]. Reports from multiple countries indicated a near-elimination of influenza and RSV circulation during 2020, followed by an atypical resurgence of RSV following the relaxation of NPIs in 2021 [8,9].

The re-emergence of RSV, often outside its traditional seasonal window, has been widely reported in regions such as Australia, Europe and North America [10-12]. Similar trends have been observed for HRV and other endemic respiratory viruses, raising concerns about their clinical impact on susceptible populations [13,14]. In parallel, the continuous evolution of SARS-CoV-2, marked by the emergence of new variants of concern (VOCs) such as Gamma and Delta, has added complexity to the respiratory virus landscape [15,16]. In Brazil, the

epidemiological dynamics of respiratory viruses during 2021 reflected this complex interplay between pandemic control measures, SARS-CoV-2 variant dissemination and the reintroduction of other respiratory pathogens. The national respiratory virus surveillance network, supported by the Ministry of Health, expanded molecular testing capacity and sequencing efforts, particularly for SARS-CoV-2 [17-19]. Despite these efforts, limited data exist on the comparative clinical and epidemiological profiles of SARS-CoV-2 variants and other respiratory viruses circulating during this period, especially outside large urban centers.

Integrated respiratory virus surveillance has gained increasing relevance as a tool for the timely detection of outbreaks, monitoring of viral evolution and assessment of healthcare system burden [20,21]. Understanding age distribution, clinical severity and hospitalization risk associated with different respiratory viruses is essential for guiding clinical management and informing public health policies [22,23].

Furthermore, the unusual resurgence of HRV in some regions, associated with higher hospitalization rates among adults, has highlighted the need for comprehensive viral surveillance strategies that go beyond SARS-CoV-2 monitoring [24,25]. Investigating the clinical behavior of HRV, along with other viruses such as influenza and RSV, in the post-pandemic context is critical to identify potential shifts in disease burden and healthcare demands [26,27].

Although the burden of SARS-CoV-2 has declined with widespread vaccination and prior immunity, a snapshot of the acute phase of the pandemic remains pertinent to current respiratory-virus management. Multiple pathogens with

overlapping symptomatology continue to co-circulate, reinforcing the need for integrated, multiplex surveillance and diagnostics beyond a single pathogen [28-31]. The pandemic period profoundly altered pre-existing seasonality (e.g. suppression of influenza followed by atypical rebounds; altered RSV waves), underscoring how clinical presentation alone is insufficient for accurate etiologic attribution [32,33]. Documenting co-circulation and variant-specific patterns during 2021 can provide an evidence base for sustained sentinel, laboratory-supported surveillance in the post-pandemic setting [28,29,34].

Given these knowledge gaps, the present study aims to describe the epidemiological, clinical and hospitalization profiles of patients with laboratory-confirmed respiratory viral infections diagnosed in 2021, comparing SARS-CoV-2 variants, influenza, RSV, HRV and virus-negative cases within an integrated respiratory virus surveillance framework in Brazil.

2. Objectives

To describe the epidemiological, clinical and hospitalization profiles of patients with laboratory-confirmed respiratory viral infections diagnosed in 2021, comparing SARS-CoV-2 (including variants), influenza, respiratory syncytial virus, human rhinovirus and negative cases within the framework of an integrated respiratory virus surveillance system during the COVID-19 pandemic.

3. Materials and Methods

This retrospective study involved the laboratory evaluation of respiratory samples collected throughout 2021. Samples were obtained as part of a community service project for SARS-CoV-2 detection during the COVID-19 pandemic. This

initiative was conducted by the Federal University of Juiz de Fora in partnership with the Juiz de Fora City Hall to provide molecular diagnosis of COVID-19. The Institute of Biological Sciences participated in this initiative by making the Center for Microbiology Studies (CEMIC) available for testing.

3.1. Ethical Approval

This study was approved by the Research Ethics Committee on Human Beings under protocol number 70133723.3.0000.5147. Anonymity was not fully assured because limited personal identifiers were required during data collection and verification procedures; however, confidentiality was strictly maintained. All records were de-identified and assigned a unique study identification code before analysis. The linkage key (code-to-identity file) was stored separately from the analytical dataset and was accessible only to the principal investigator and designated authorized personnel. Electronic data were stored on password-protected, access-restricted institutional computers/servers, with regular backups. Files containing sensitive information were additionally protected through restricted folder permissions and, when available, encryption. No identifiable information is disclosed in any publication resulting from this study.

The local ethics committee waived the requirement for informed consent because the study was conducted as a diagnostic action offered to the entire city population, and personal data were anonymized by the study coordinators.

3.2. Clinical sample collection

Nasal and nasopharyngeal swab samples were collected as part of a pandemic response initiative organized by the Juiz de Fora city government in partnership with the Federal University of Juiz de Fora. This initiative aimed to test patients

treated across the city's public and private healthcare facilities. Patient inclusion in this study followed the COVID-19 diagnostic manual established by the Minas Gerais State Health Department, which provided standardized guidance on case definitions, clinical syndromes, sample collection, storage, transportation and laboratory testing of suspected cases [35].

In addition, the State Health Secretariat established a clinical and operational pathway to determine which patients met the criteria for diagnostic testing, as follows: (i) individuals with influenza-like illness presenting dyspnea or respiratory discomfort, persistent chest pressure or pain, oxygen saturation ≤94% on room air, bluish discoloration (cyanosis) of the lips or face, or clinical worsening of underlying conditions; and (ii) suspected cases whose samples were collected between the 3rd and 5th day after symptom onset [36]..

All samples included in this study were collected between February and September 2021. Samples that had previously tested positive for SARS-CoV-2 with high viral loads were selected, as next-generation sequencing is more efficient and yields more reliable results in such cases.

3.3. Data Collection

Notification forms were completed by healthcare professionals at the time of sample collection. These forms contained clinical and epidemiological information, signs and symptoms at the time of sample collection, comorbidities, and hospitalization status. All healthcare professionals involved in collecting samples and information were trained in accordance with state health department guidelines. In this study the term hospitalized referred to patients with a documented clinical indication for inpatient admission at the time of enrollment.

Access to patient follow-up data was not possible due to clinical management policies, restricted access to medical records and the decentralized nature of patient referral and discharge pathways. This constraint represents a limitation of the study, inherent both the design and the characteristics of participants recruited through a public diagnostic service.

3.4. Laboratory Testing

Viral RNA was extracted using the PureLink™ RNA Mini Kit (Thermo Fisher Scientific), following the manufacturer's instructions. The isolated RNA was stored at - 80°C. Detection reactions for the studied viruses (SARS-CoV-2, influenza A, influenza B, RSV and HRV) were performed by RT-qPCR using two different platforms: TaqMan and SYBR Green. SARS-CoV-2, influenza A and B, and RSV were detected using commercial kits (Thermo Fisher Scientific and Viasure). HRV detection followed the protocol described by Martins et al [37].

3.5. Complete Genome Sequencing of SARS-CoV-2

The SARS-CoV-2 genome was subjected to high-throughput sequencing using Nanopore technology. Previously published genomes were used as references for molecular epidemiological analyses (phylogeny, conservation and genetic alterations) of the circulating viral lineages in Juiz de Fora and neighboring cities. Sequencing reactions were performed using the MinION® platform (Oxford Nanopore Technologies). All sequencing steps were conducted according to the manufacturers' guidelines for both equipment and reagents, following these stages: (i) RNA extraction; (ii) amplicon preparation; (iii) genomic library preparation and adapter ligation; and (iv) loading the library onto the flow cell.

After obtaining raw sequences, genome assembly and editing were performed using Geneious® software.

For the bioinformatics analysis, starting from the raw .fast5 files generated by MinION (Oxford Nanopore Technologies) sequencing, basecalling was performed using Guppy Basecalling Software (Oxford Nanopore Technologies, version 6.0.6) in fast mode. Filtered .fastq files were then demultiplexed using Guppy_Barcoder (part of the Guppy Basecalling Suite, version 6.0.6), considering all 23 barcodes assigned to samples during library preparation. After demultiplexing by barcode, a read-length filtering step and quality control were conducted using the ARTIC Network bioinformatics toolkit, available on GitHub [38]. Length filtering was performed with Guppyplex, following the ARTIC consortium's recommendations to mitigate the inclusion of chimeric reads, using a length range between 400 and 700 base pairs.

The length-filtered .fastq files for each barcode were concatenated, and the ARTIC MinION pipeline was executed to generate consensus FASTA sequences. These consensus genomes were then used as input for downstream clade analyses.

The Nextclade web tool (version 2.2.0) was used to compare the sequences obtained in this study against SARS-CoV-2 reference sequences, assign clades and determine their positions within the global SARS-CoV-2 phylogenetic tree [39]. The Auspice web-based visualization tool (version 0.2.0), an open-source platform, was used to visualize the phylogenetic trees generated by Nextclade.

Additionally, Pangolin (Phylogenetic Assignment of Named Global Outbreak Lineages) version 4.1.1 for Linux was used in command-line mode (UShER

mode), with a maximum ambiguity threshold of 0.3, to assign each SARS-CoV-2 genome sequence from this study to its most likely Pango lineage based on the reference SARS-CoV-2 dataset [40].

3.6. Statistical analysis and data treatment

Descriptive statistics were used to summarize the data. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Age comparisons between groups were conducted using the Kruskal-Wallis test. Logistic regression analysis was used to assess predictors of hospitalization, risk factor and infection severity. Risk factors were coded as binary variables (0 = absence, 1 = presence) and missing data were treated as absence (zero). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from 2 x 2 contingency tables for each risk factor. Statistical significance was set at a p-value threshold of 0.05. All analyses were performed using SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA) and Python, employing the pandas, SciPy and statsmodels libraries.

Clinical severity was defined as the presence of dyspnea/respiratory discomfort or oxygen saturation (SpO_2) <95% on room air at the time of diagnostic assessment. The definition follows Brazilian public health guidance for influenza-like illness/ severe acute respiratory infection (SARI) and COVID-19 and is concordant with international guidance that considers SpO_2 <94% as severe illness[41-43]. For transparency, "respiratory discomfort" encompassed both patient-reported increased work of breathing (e.g., dyspnea, orthopnea, chest tightness) and objective signs of respiratory distress, including tachypnea for age, use of accessory muscles, nasal flaring, intercostal retractions and inability to

complete full sentences, as documented by the evaluating clinician [41,44]. Hospitalization, Intensive Care Unit (ICU) admission and death were analyzed as separate clinical outcomes and did not upstage the baseline severity classification.

3.7. Risk factor analysis

Associations between baseline risk factors and laboratory-confirmed infection were evaluated using logistic regression, with test-negative controls as the comparator group. The primary outcome was SARS-CoV-2 (any). Secondary outcomes were modeled by virus (influenza B, RSV, HRV) and, for SARS-CoV-2 variants (Gamma and Delta). Univariate models included a single predictor; multivariable models prespecified mutual adjustment among comorbidities available in the surveillance worksheet (diabetes, obesity, cardiac disease). If additional demographic and contextual covariates (age, sex and seasonality) become available, these will be incorporated into fully adjusted models. In analyses referring to individual viruses, SARS-CoV-2 was not treated as a single aggregate category; instead, it was partitioned into its main circulating variants (Gamma and Delta). Accordingly, contrasts for SARS-CoV-2 are presented both as an aggregate (any SARS-CoV-2 vs test-negative) and as variant-specific models (Gamma vs test-negative; Delta vs test-negative).

4. Results

4.1. Baseline characteristics

A total of 337 patients (one sample per patient) were included in the study. The demographic characteristics of the study population are presented in Table 1.

Table 1. Demographic data of patients included in the study

	General	Positive (%)	Negative (%)	SC2 (%)	Flu B (%)	RSV (%)	HRV (%)	Coinfection (%)
Female	180 (53.4)	128 (54.7)	52 (50.5)	77 (56.2)	2 (50.0)	12 (44.4)	31 (53.4)	6 (75.0)
Male	157 (46.6)	106 (45.3)	51 (49.5)	60 (43.8)	2 (50.0)	15 (55.6)	27 (46.6)	2 (25.0)
Total	337 (100)	234 (100)	103 (100)	137 (100)	4 (100)	27 (100)	58 (100)	8 (100)
Age								
Mean	48.3	47.4	50.4	55.8	56.3	25.8	40.7	20.0
Median	51.0	51.5	51	59.0	64.5	4.0	40.5	22.5
SD	24.4	24.7	23.8	17.1	20.4	30.7	27.4	17.5
P		0.2998		<0.001				

SARS-CoV-2 (SC2), influenza B (FluB), respiratory syncytial virus (RSV), human rhinovirus (HRV), Standard Deviation (SD) and p-value (P)

4.2. Positivity profile by virus and age distribution

A total of 337 respiratory samples were analyzed during the study period. The overall positivity rate for respiratory viruses was 69.4%, with SARS-CoV-2, HRV, RSV and influenza B being the most frequently detected viruses. The Gamma variant of SARS-CoV-2 accounted for 23.4% of all cases, followed by HRV (17.2%), RSV (8.0%), Delta variant (7.1%) and influenza B (3.6%). Negative results represented 30.6% of the total samples. Coinfections such as RSV/HRV (2.4%) and influenza B/RSV (1.8%) were also identified. Temporal patterns of SARS-CoV-2 variants and overall respiratory virus circulation during 2021 are shown in Figure 1 and Figure 2, respectively.

The mean, median, and standard deviation for the time from symptom onset to sample collection for the entire study population were 8.15, 4.0 and 60.25 days, respectively. For positive samples, these times were 4.94, 4.0 and 5.07 days (mean, median and standard deviation), and for negative samples, they were 4.6, 4.0, and 2.78 days (mean, median, and standard deviation), respectively.

Age distribution varied by virus, with RSV predominantly affecting young children (mean, median and standard deviation: 25.8, 4.0 and 30.7 years), and SARS-CoV-2 and influenza B showing higher median ages (59.0 and 64.5 years, respectively). Influenza B and SARS-CoV-2 cases had a higher median age among hospitalized patients (p = 0.021). Kruskal-Wallis tests revealed a significant difference in age distribution between virus groups (p<0.001).

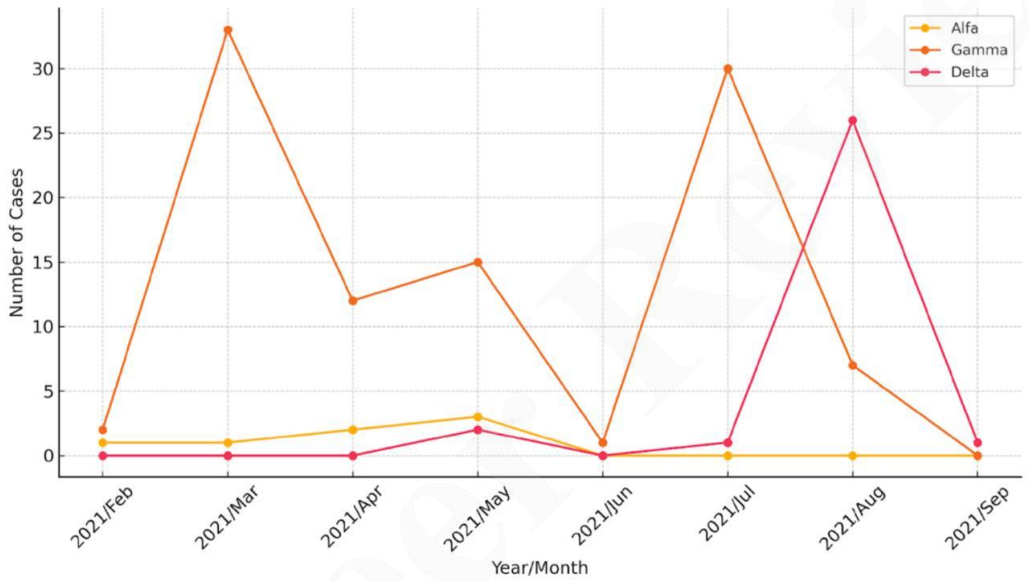

Figure 1. Seasonality by SARS-CoV-2 variants (2021). The figure shows the monthly distribution of different SARS-CoV-2 variants during 2021.

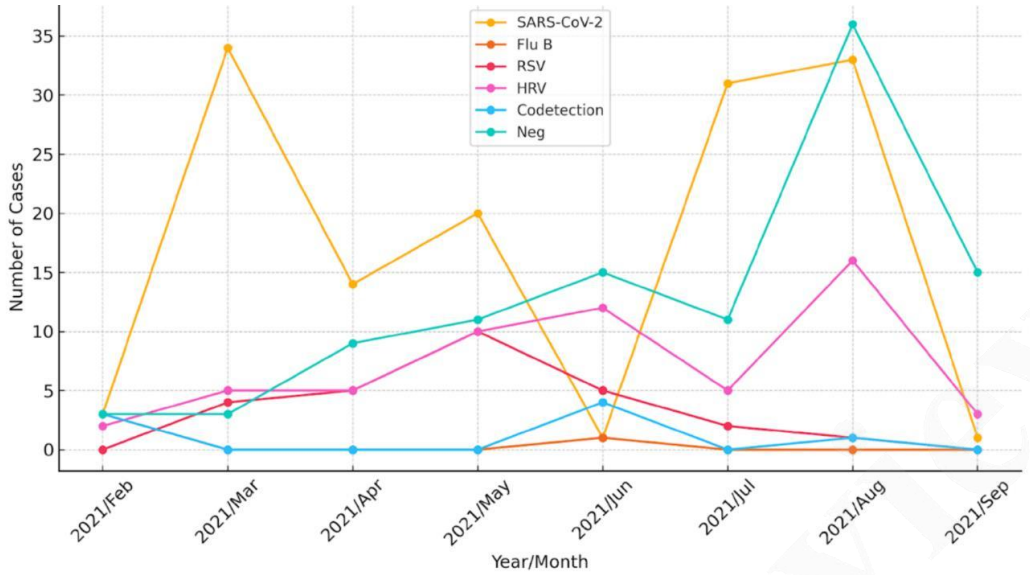

Figure 2. Seasonality of respiratory viruses with SARS-CoV-2 grouped (2021). Monthly distribution of respiratory viruses detected during 2021, showing SARS-CoV-2 grouped with other viruses. Legend abbreviations: influenza B virus (Flu B); respiratory syncytial virus (RSV); human rhinovirus (HRV) and negative cases (Neg)

4.3. Symptom profile and principal component analysis (PCA)

The distribution of reported symptoms varied across viral groups. For SARS-CoV-2 (any, n = 136), the most frequent symptoms were cough (79%; 106/135) versus 69% (70/102) in test-negative controls, and fever (64%; 87/136) versus 35% (36/102). For HRV (n=58), cough occurred in 72% (41/57) versus 69% (70/102) among controls, and sore throat in 39% (22/57) versus 38% (38/101). For influenza B (n=4), dyspnea occurred in 75% (3/4) versus 33% (33/101), and oxygen saturation <95% in 75% (3/4) versus 29% (29/101). For RSV (n=26), cough occurred in 69% (18/26) versus 69% (70/102), and fever in 52% (13/25) versus 35% (36/102). Denominators differ across symptoms due to missing data. Percentages were calculated using the number with available data for that symptom.

PCA demonstrated distinct clustering patterns, with PC1 and PC2 explaining 93.9% of the total variance. The biplot and heatmap highlighted how different symptoms contributed to discrimination among viruses. The PCA results are summarized in Figure 3 (PCA biplot) and Figure 4 (symptom frequency heatmap).

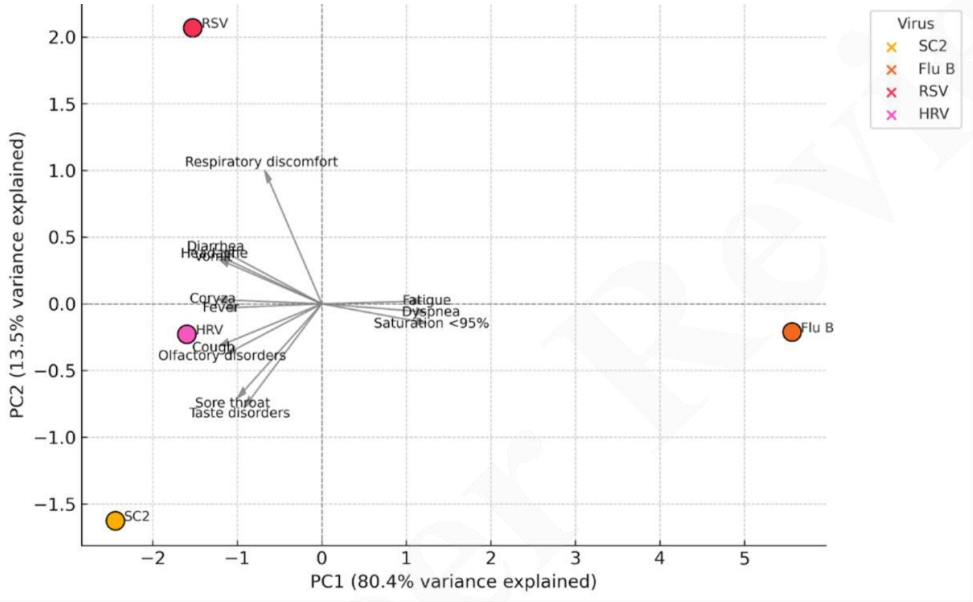

Figure 3. PCA biplot of symptom profiles. The biplot shows the projection of respiratory viruses (SARS-CoV-2, influenza B, RSV and HRV) in the two-dimensional space defined by the first two principal components (PC1 and PC2), which together explain 93.9% of the total variance (PC1: 80.4%; PC2: 13.5%). The variable vectors represent the contribution of each symptom to the components. Influenza B virus (Flu B); respiratory syncytial virus (RSV) and human rhinovirus (HRV)

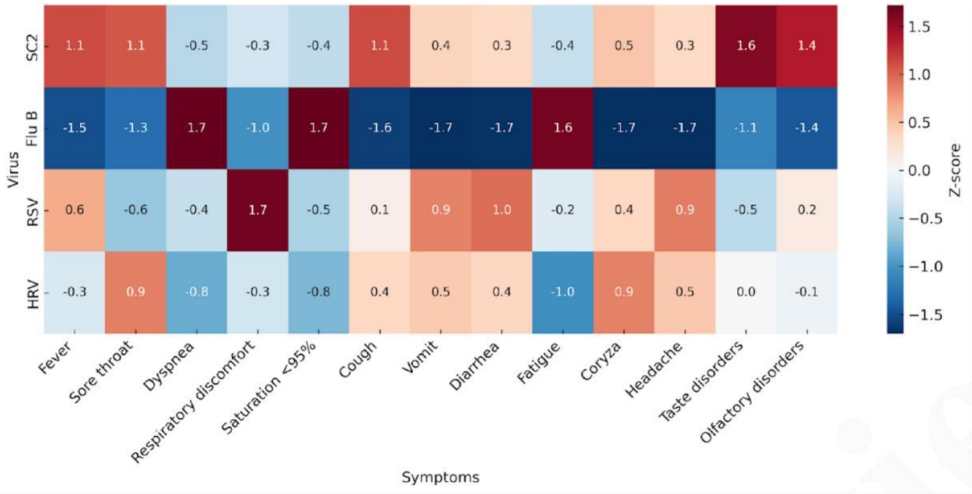

Figure 4. Symptom frequency heatmap for respiratory viruses. The heatmap presents the standardized z-score distribution of symptom frequencies across the four viruses (SARS-CoV-2, influenza B, RSV and HRV). Red shades indicate higher-than-average symptom frequencies, whereas blue shades represent lower-than-average frequencies. Influenza B virus (Flu B); respiratory syncytial virus (RSV) and human rhinovirus (HRV)

4.4. Clinical severity based on symptoms and hospitalization (including SARS-CoV-2 variants)

Severity was defined by the presence of dyspnea, respiratory discomfort or oxygen saturation below 95%. When SARS-CoV-2 variants were compared, the Gamma variant showed approximately threefold higher odds of severe symptoms than the Delta variant (OR ≈ 3.0; 95% CI: 1.21–7.32). Differences between Alpha versus Gamma and Alpha versus Delta were not statistically significant.

When all viruses, including SARS-CoV-2, were compared, no significant differences were observed in the distribution of severe symptoms.

Analyses of hospitalization and ICU admission analysis also showed higher odds for Gamma compared whith DELTA, but global chi-square tests across variants did not reach statistical significance (Figure 5).

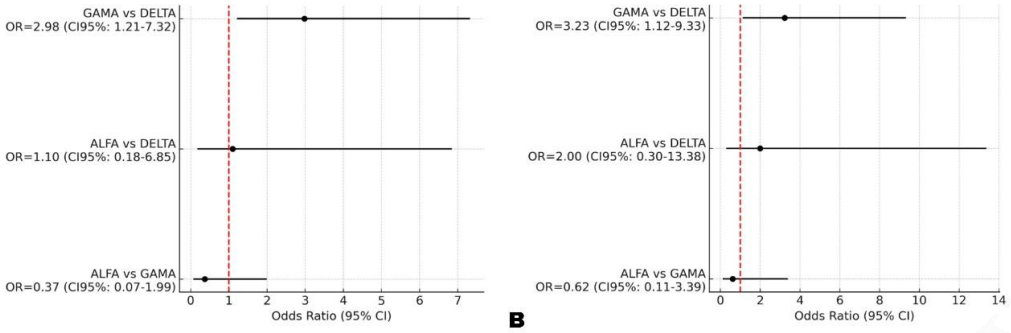

Figure 5. **A.** Odds ratios for severe clinical outcomes according to SARS-CoV-2 variants. Comparison of the frequency of severe respiratory symptoms (dyspnea, respiratory discomfort, or oxygen saturation <95%) among patients infected with Gamma, Delta, and Alpha variants. The comparison between Gamma and Delta showed an odds ratio (OR) of approximately 3.0 (95% CI: 1.21–7.32), indicating statistical significance (p = 0.0256). **B**. Hospitalization and ICU admission according to SARS-CoV-2 variants. Analysis of hospitalization and ICU admission rates among patients infected with different SARS-CoV-2 variants (Gamma, Delta, Alpha). Higher odds were observed for Gamma compared with Delta, but global comparisons did not reach statistical significance (p = 0.078).

4.5. Coinfection Analysis

No significant association was found between coinfection and increased clinical severity (p = 0.298), hospitalization (p = 0.267) or ICU admission (p = 0.603).

4.6. Risk Factors Associated with COVID-19 Infection

Diabetes (p = 0.0118) and obesity (p = 0.0115) were significantly associated with COVID-19 infection. Patients with diabetes had 2.14 times higher odds (95% CI: 1.21–3.79), and those with obesity had 7.02 times higher odds (95% CI: 1.49–33.00) of COVID-19 infection compared with non-COVID-19 cases.

4.7. Risk Factors by Virus – Univariate and Multivariate Logistic Regression

No significant associations were observed between specific risk factors and infection by individual viruses in univariate analyses. Multivariable logistic regression identified only one significant result: cardiac disease was associated

with Gamma infection (adjusted OR: 3.54; 95% CI: 1.99–6.29; p < 0.0001). Associations are interpreted as differences in SARS-CoV-2 test positivity among symptomatic individuals undergoing testing, rather than as population-level susceptibility to infection.

4.8. Risk factors for infection (aggregate vs variant-specific)

In the primary aggregate model (SARS-CoV-2 [any] vs test-negative), diabetes and obesity were associated with infection (Table 2). When SARS-CoV-2 was analyzed by variant, cardiac disease was associated with Gamma infection in multivariable models, whereas the aggregate SARS-CoV-2 model did not show a significant association with cardiac disease. This divergence is compatible with variant-level heterogeneity and with differences in confounding structure and case-mix across epidemic phases. Complementary results for influenza B, RSV, and HRV are provided in Supplementary Tables 3-6.

4.9. Stability of multivariable estimates.

Some multivariable cells exhibited instability due to sparse data and quasi-separation (notably for obesity in certain outcomes). To avoid misleading magnitudes, these entries are shown as dashes ("—"), with a table footnote indicating non-convergence/instability.

Table 2. Risk factors for SARS-CoV-2 infection, aggregate and by variant.

	SARS-CoV-2		Gamma (SARS-CoV-2)		Delta (SARS-CoV-2)	
Predictor	Univariate OR (95% CI); p	Multivariable OR (95% CI); p	Univariate OR (95% CI); p	Multivariable OR (95% CI); p	Univariate OR (95% CI); p	Multivariable OR (95% CI); p
Diabetes	1.34 (0.66–2.71); p=0.413	1.24 (0.58–2.63); p=0.582	1.33 (0.63–2.79); p=0.456	—	1.08 (0.37–3.16); p=0.887	1.91 (0.55–6.61); p=0.306
Obesity	10.14 (0.58–177.57); p=0.051	—	9.16 (0.50–166.74); p=0.071	—	10.52 (0.46–240.72); p=0.071	—
Cardiac disease	1.42 (0.75–2.71); p=0.282	1.30 (0.66–2.56); p=0.440	2.00 (1.02–3.90); p=0.041	—	0.47 (0.14–1.54); p=0.205	0.39 (0.10–1.47); p=0.163

Univariate = single predictor; multivariable = mutual adjustment among diabetes, obesity, cardiac disease. Full adjustment (age, sex, vaccination, calendar period, region) will be added when available. Software: Python 3.11.8 (pandas 1.5.3; NumPy 1.24.0; statsmodels 0.13.5). Cells marked ("—") indicate non-convergence or instability due to sparse data (quasi-separation); in these instances, standard maximum-likelihood logistic regression is unreliable. If desired, penalized logistic regression (e.g., Firth or L2 ridge) can be used to obtain finite, more stable estimates.

5. Discussion

The distribution of respiratory viruses across age groups revealed virus-specific patterns, with RSV predominantly affecting younger children (median age 2 years), whereas SARS-CoV-2 and influenza B were more frequently detected among older age groups. These findings align with well-documented epidemiological profiles of these pathogens. RSV is classically associated with early childhood infections and seasonal bronchiolitis outbreaks, whereas influenza and SARS-CoV-2 have broader age ranges of susceptibility and severity [1,45,46].

However, it is important to recognize potential testing biases that may have influenced the observed age distribution, particularly for SARS-CoV-2. Several studies have demonstrated that asymptomatic or oligosymptomatic infections are more frequent among children and adolescents, which may lead to underrepresentation of these groups in surveillance systems that prioritize testing based on symptomatology [47,48]. A meta-analysis of more than 38 studies

showed that up to 46.5% of SARS-CoV-2 infections in children were asymptomatic, compared with significantly lower rates among older adults.[44] Similarly, epidemiological reports from the United States during different phases of the pandemic indicated a decreasing median age of COVID-19 cases as younger populations became more involved in community transmission [49,50]. These findings underscore the importance of considering detection bias when interpreting age-related patterns of viral positivity. Moreover, population-based modeling studies suggest that young adults (ages 20–49 years) have played a central role in sustaining transmission of SARS-CoV-2, often preceding increases in incidence among older adults by several days [51]. These dynamics may not be fully captured in datasets restricted to symptomatic individuals, highlighting the value of integrated surveillance systems that incorporate asymptomatic testing and population sampling.

Therefore, while the age profiles observed in the present study are consistent with known clinical and epidemiological features of respiratory viruses, caution is needed when attributing differences solely to biological susceptibility. Underlying testing strategies and symptom-driven case definitions likely contribute to these distributions and should be accounted for future surveillance designs.

PCA was performed to explore the distribution of clinical symptoms among cases of SARS-CoV-2, influenza B, RSV and HRV. The analysis revealed clustering patterns consistent with virus-specific symptom profiles. For example, anosmia and ageusia contributed significantly to the SARS-CoV-2 cluster, whereas nasal congestion and rhinorrhea were more strongly associated with HRV and RSV. These findings are supported by previous studies that used PCA or clustering methods to identify clinical phenotypes in COVID-19 and other respiratory

infections [52,53]. The ability of PCA to distinguish symptom patterns has proven useful not only for characterizing disease manifestations but also for inferring potential prognostic differences [52].

Importantly, our data highlight an important challenge in respiratory virus surveillance: the overlap in clinical presentation between SARS-CoV-2 and other respiratory pathogens, especially during periods of co-circulation. Several studies have demonstrated that the clinical symptomatology of influenza, RSV and SARS-CoV-2, particularly with Omicron and later variants, has become increasingly indistinguishable [54,55]. This overlap may contribute to clinical diagnostic confusion, particularly in syndromic surveillance systems or in resource-limited settings where multiplex testing is unavailable. In a pediatric cohort study, initial clinical diagnoses based on symptoms alone often failed to accurately distinguish among the three main viruses [56].

These findings support the importance of integrated respiratory surveillance combining molecular diagnostics with clinical data. PCA and similar multivariate approaches can enhance the interpretation of symptom-based screening but should be complemented by laboratory testing to avoid misclassification, especially in the early phases of a pandemic context, when novel viruses may co-circulate with endemic pathogens. The circulation of other respiratory viruses during the peak of the COVID-19 pandemic in our study region may have confounded clinical identification of true SARS-CoV-2 cases, reinforcing the limitations of syndromic surveillance alone.

The year 2021 marked a transition from SARS-CoV-2 dominated transmission to the re-emergence of endemic respiratory viruses. Before 2020, RSV and

influenza exhibited predictable winter seasonality and stringent non-pharmaceutical interventions during the acute pandemic phase suppressed these viruses. As measures relaxed, out-of-season rebounds and immunity gaps were documented across multiple settings [32,33]. Within this context, overlapping symptom profiles across SARS-CoV-2, RSV, influenza and HRV complicate syndromic diagnosis, supporting continued use of multiplex molecular testing within integrated surveillance frameworks [28,29,30,31]. Moreover, variant-level heterogeneity, exemplified in Brazil by more severe infection patterns during the Gamma period, indicates that aggregate “SARS-CoV-2” effects can obscure clinically meaningful differences across pandemic phases [57,58].

Infection with the Gamma (P.1) variant of SARS-CoV-2 was significantly associated with increased clinical severity compared to Delta, with Gamma-infected patients exhibiting approximately three-fold higher odds of presenting severe symptoms, such as dyspnea, respiratory discomfort, or oxygen saturation < 95%, even after adjusting for age and comorbidities. Although no significant differences were observed between Alpha, Gamma, or Alpha-Delta comparisons, the higher severity associated with Gamma aligns with epidemiological data from Brazil showing increased mortality and younger age distribution during the P.1 circulation [15,59,60].

The Gamma variant has been reported to carry a higher viral load, up to tenfold higher than that of earlier lineages, as well as enhanced transmission capacity (1.7–2.4 times more transmissible) and partial evasion of pre-existing immunity from prior infection or vaccination [15,61]. These virological characteristics likely contributed to the greater clinical severity observed in our cohort and may have disproportionately affected younger, unvaccinated individuals, an

epidemiological pattern consistent with the heightened disease burden reported in the states of Amazonas and São Paulo [15,59,62]. Despite these concerning attributes, Gamma failed to achieve sustained global dominance comparable to Delta or Omicron. Several factors may explain this limited global spread. First, competitive disadvantage: Gamma emerged in Brazil but was quickly displaced by more transmissible variants such as Delta, which possessed greater fitness in the global epidemiological landscape [63,64]. Second, immune-evasion trade-offs: although Gamma harbored immune-escape mutations like E484K, its balance of transmissibility and immune escape may have been insufficient to outcompete other VOCs in regions with higher vaccination or prior exposure [65,66]. Third, genetic bottlenecks and founder effects: Gamma's emergence was geographically concentrated, and without early introductions into multiple global hubs, its spread was inherently limited compared to Alpha or Delta [66].

While Gamma's virological properties probably contributed to more severe disease during its period of regional dominance, its international spread was constrained by rapid replacement with fitter variants [67]. This situation underscores the need for real-time genomic surveillance and variant-specific clinical preparedness, particularly in settings experiencing concurrent circulation of multiple SARS-CoV-2 genetic variants.

Importantly, our data also suggest that the clinical severity observed was not solely driven by host comorbidities, as multivariable analysis identified cardiac disease as the only individual risk factor significantly associated with Gamma infection. These results emphasize the role of variant-specific viral characteristics in shaping disease presentation.

Given the high burden associated with Gamma during the circulation period, the incorporation of variant-based risk stratification into surveillance and clinical management protocols may be crucial, especially in resource-limited settings where early identification of high-risk patients can improve outcomes.

Coinfections involving respiratory viruses, such as RSV/HRV and influenza B/RSV, were detected at low frequency and did not demonstrate statistically significant associations with clinical severity, hospitalization or ICU admission. These findings suggest that, within our population and study period, coinfection was not a major determinant of adverse clinical outcomes.

Although some previous studies have reported that viral coinfections may exacerbate disease severity or prolong illness duration, other studies have not identified evidence supporting this association, particularly in community-based or non-hospitalized populations [68-72]. Our results align with this latter body of evidence, indicating that the presence of more than one respiratory virus does not necessarily imply a more severe clinical course.

Monitoring coinfection patterns remains important, especially in pediatric or immunocompromised patients and in future epidemics in which new variants or pathogens may interact differently. Larger sample sizes and comprehensive multiplex diagnostic approaches may be necessary to fully elucidate the clinical implications of viral coinfection [69,73].

The comorbidities diabetes and obesity were significantly associated with SARS-CoV-2 infection when compared with virus-negative cases. Patients with diabetes had more than twice the odds of testing positive for COVID-19, whereas those with obesity had more than seven times the odds. These findings reinforce the

well-established relationship between metabolic disorders and increased susceptibility to SARS-CoV-2 infection [74].

Multiple studies have demonstrated that obesity and diabetes not only increase the risk of infection but also contribute to more severe disease progression, possibly due to chronic low-grade inflammation, impaired immune responses and higher expression of ACE2 receptors in adipose tissue [75]. Moreover, metabolic comorbidities have been associated with prolonged viral shedding and greater viral replication, which may also increase the likelihood of a positive RT-qPCR result [76,77].

In risk-factor models, obesity and diabetes were associated with SARS-CoV-2 test positivity among symptomatic individuals [78]. By contrast, severity models had smaller effective samples sizes and different confounding structures (e.g., differences across epidemic phases), which may have limited the power to detect consistent associations between these comorbidities and several outcomes. Thus, the observed contrast reflects differences in target measures (positivity vs severity), statistical power and patient characteristics.

While many investigations have focused on the role of these conditions in worsening clinical outcomes, our findings indicate that these comorbidities were associated with a higher likelihood of SARS-CoV-2 test positivity among symptomatic individuals who presented for testing. This emphasizes the importance of prioritizing individuals with these comorbidities in surveillance, testing strategies and vaccination campaigns.

In multivariable analyses evaluating virus-specific risk factors, cardiac disease emerged as the only significant clinical condition associated with infection by the

Gamma variant of SARS-CoV-2. Patients with pre-existing cardiac comorbidities had more than three times the odds of testing positive for Gamma, even after adjusting for confounding variables. This association underscores the potential interaction between host vulnerability and variant-specific pathogenic features.

Cardiovascular disease has consistently been identified as a key risk factor for both susceptibility to infection and adverse clinical outcomes in COVID-19, possibly due to chronic endothelial dysfunction, systemic inflammation and upregulation of ACE2 expression [79,80]. Our findings suggest that these vulnerabilities may be particularly relevant in the context of more virulent variants such as Gamma.

While other comorbidities such as diabetes and obesity were associated with COVID-19 infection overall, their lack of significance in virus-specific models further emphasizes the unique role of cardiac disease in shaping susceptibility to Gamma. This result highlights the need for tailored surveillance and protective measures targeting individuals with cardiovascular comorbidities, especially during periods of circulation of more pathogenic viral lineages.

A standardized 2021 protocol, implemented in a mid-sized regional setting and integrating multi-virus testing (SARS-CoV-2/RSV/influenza/HRV), variant-aware analyses and quantitative symptom profiling PCA, strengthens the internal validity and operational relevance of the study.

The present study intentionally focused on a harmonized 2021 workflow to reduce bias from evolving testing indications and data capture across years. Accordingly, a formal pre-/post-pandemic comparison was not attempted within the same analytic framework. Temporal generalizability beyond 2021 should, therefore, be

interpreted with caution. Nevertheless, the core inferences highlighted here: variant-specific clinical patterns and the diagnostic implications of multivirus co-circulation, directly inform present-day priorities in integrated sentinel surveillance and clinical screening [28-31,34]. Because our analyses were restricted to symptomatic individuals who were referred to the molecular diagnostic service for acute respiratory disease, some degree of ascertainment or spectrum bias is possible, particularly the under-representation of asymptomatic or minimally symptomatic infections in the general population. Consequently, the data presented here do not allow inferences about population-level biological susceptibility to infection, especially given the limited variant-specific subgroups, which may reduce the precision of broader epidemiological estimates. In addition, the cross-sectional nature of the evaluation, the absence of longitudinal outcomes, the relatively small variant-specific sample sizes and the lack of certain contextual covariates in some models may constrain generalizability. Despite these limitations, the study design and the originality of the dataset offer relevant insights and underscore the need for broader prospective studies to achieve more comprehensive population coverage.

6. Conclusion

This study highlights the importance of integrated respiratory virus surveillance systems capable of detecting multiple etiologic agents simultaneously. By comparing SARS-CoV-2 (including its variants) with influenza B, RSV, HRV, and virus-negative cases, we were able to characterize distinct clinical and epidemiological patterns that would likely have been overlooked in SARS-CoV-2, exclusive monitoring programs. Our findings emphasize that respiratory virus

circulation during the pandemic was not limited to SARS-CoV-2. Other pathogens, particularly HRV and RSV, showed relevant detection rates and contributed to the clinical burden, underscoring the limitations of symptom-based diagnosis in periods of viral co-circulation. The similarity in symptom profiles across viral etiologies, demonstrated through PCA, further supports the need for molecular diagnostics to inform accurate case definitions and public health responses. Importantly, infection with the Gamma variant was significantly associated with increased clinical severity and with cardiac comorbidities, reinforcing the need for variant-specific risk stratification. In contrast, coinfections were not associated with greater severity, although ongoing monitoring remains essential, particularly in vulnerable populations. The identification of diabetes and obesity as risk factors for SARS-CoV-2 infection, and of cardiac disease as a specific predictor for Gamma infection, reinforces the interplay between host vulnerability and pathogen behavior. These findings support the prioritization of individuals with metabolic and cardiovascular comorbidities in testing strategies and clinical management. Implementation efforts should prioritize multiplex PCR at sentinel primary care and emergency sites, standardized case definitions and minimal clinical datasets, rapid electronic reporting with weekly integrated bulletins, and routine genomic sequencing of a subset of positives samples to track variant dynamics. Local workflows should emphasize turnaround time, linkage of laboratory and clinical data, and feedback loops to inform public health authorities. In summary, our results validate the relevance of multiplex diagnostic approaches and genomic surveillance to accurately monitor respiratory virus circulation, differentiate clinical presentations and guide evidence-based public health decisions in the post-pandemic era.

7. Conflict of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

8. Author contributions

Conceptualization: CGD, KO, ASAW; Methodology: KO, ASAW, ACC, VGF; Formal analysis: KO, ASAW. CGD; Investigation: KO, CGD, ASAW, EMAC, ABFM, VCD, VLS; Data curation: KO, ASAW, CGD, VGF, ACC; Visualization: KO, ASAW, CGD, EMAC; Writing – original draft: KO; Writing – review & editing: CGD, KO, ASAW; Supervision: CGD; Project administration: CGD, KO; Funding acquisition: CGD.

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10. Ethics declaration

This study was approved by the Research Ethics Committee on Human Beings under protocol number 70133723.3.0000.5147. Anonymity was not assured

because limited personal identifiers may be required during data collection and verification procedures. Nevertheless, confidentiality was strictly maintained. All records were de-identified and assigned a unique study identification code before analysis. The linkage key (code-to-identity file) was stored separately from the analytical dataset and was accessible only to the principal investigator and designated authorized personnel. Electronic data were stored on password-protected, access-restricted institutional computers/servers, with regular backups. Files containing sensitive information were additionally protected through restricted folder permissions and, when available, encryption. No identifiable information is disclosed in any publication resulting from this study.

Generative AI tools were not used for literature review, study design, data collection, data analysis, interpretation of results, manuscript drafting, editing, figure creation, or reference management. This research has not been previously presented, published, or disseminated in any form or through any platform.

11. Article Highlights

- Integrated respiratory virus surveillance in Brazil during 2021 identified substantial co-circulation of SARS-CoV-2, RSV, HRV and influenza B among symptomatic patients (n = 337).
- PCA showed virus-specific symptom patterns, but with clinically relevant overlap across respiratory viruses, limiting syndromic differentiation without laboratory confirmation.
- SARS-CoV-2 Gamma variant (P.1) was associated with higher odds of

severe clinical presentation than the Delta variant (B.1.617.2), supporting variant-aware risk assessment.

- Diabetes and obesity were associated with increased SARS-CoV-2 test positivity among symptomatic individuals, highlighting the importance of metabolic comorbidities in clinical and surveillance prioritization.
- Viral coinfections were uncommon and were not associated with higher severity in this cohort, reinforcing that co-detection does not necessarily imply worse outcomes in sentinel testing contexts.
- Findings support routine multiplex PCR testing in sentinel settings and timely genomic characterization to inform local clinical pathways and public health responses.

12. References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Troeger C, Blacker B, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*. 2018;18(11):1191-210.
2. Iuliano AD, Roguski KM, Chang HH, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet*. 2018;391(10127):1285-300.
3. De Francesco MA, Pollara C, Gargiulo F, et al. Circulation of respiratory viruses in hospitalized adults before and during the COVID-19 pandemic in Brescia, Italy: a retrospective study. *Int J Environ Res Public Health*. 2021;18(18):9525.
4. Abo YN, Clifford V, Lee LY, et al. COVID-19 public health measures and respiratory viruses in children in Melbourne. *J Paediatr Child Health*. 2021;57(12):1886-92.
5. Obando-Pacheco P, Justicia-Grande AJ, Rivero-Calle I, et al. Respiratory syncytial virus seasonality: a global overview. *J Infect Dis*. 2018;217(9):1356-64.

6. Olsen SJ, Azziz-Baumgartner E, Budd AP, et al. Decreased influenza activity during the COVID-19 pandemic - United States, Australia, Chile, and South Africa, 2020. *Am J Transplant*. 2020;20(12):3681-5.

7. Friedrich F, Ongaratto R, Scotta MC, et al. Early impact of social distancing in response to coronavirus disease 2019 on hospitalizations for acute bronchiolitis in infants in Brazil. *Clin Infect Dis*. 2021;72(12):2071-5.

8. Eden JS, Sikazwe C, Xie R, et al. Off-season RSV epidemics in Australia after easing of COVID-19 restrictions. *Nat Commun*. 2022;13(1):2884.

9. Carvalho EMA, Ozanic K, Machado AFB, et al. Respiratory virus circulation during pandemic: why were some viruses still circulating? *Braz J Microbiol* [Internet]. 2025 [cited 2025 Jul 30]; Available from: https://link.springer.com/article/10.1007/s42770-025-01681-2.

10. Torres-Fernandez D, Casellas A, Mellado MJ, et al. Acute bronchiolitis and respiratory syncytial virus seasonal transmission during the COVID-19 pandemic in Spain: a national perspective from the pediatric Spanish Society [AEP]. *J Clin Virol*. 2021;145:105027.

11. Olsen SJ, Winn AK, Budd AP, et al. Changes in influenza and other respiratory virus activity during the COVID-19 pandemic - United States, 2020-2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(29):1013-9.

12. Foley DA, Yeoh DK, Minney-Smith CA, et al. The interseasonal resurgence of respiratory syncytial virus in Australian children following the reduction of coronavirus disease 2019-related public health measures. *Clin Infect Dis*. 2021;73(9):e2829-30.

13. Jacobs SE, Lamson DM, St George K, et al. Human rhinoviruses. *Clin Microbiol Rev*. 2013;26(1):135-62.

14. Zheng Z, Pitzer VE, Shapiro ED, et al. Estimation of the timing and intensity of reemergence of respiratory syncytial virus following the COVID-19 pandemic in the US. *JAMA Netw Open*. 2021;4(12):e2141779.

15. **Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*. 2021;372(6544):815-21.

Key contribution: Provides foundational genomic and epidemiological evidence on the emergence, spread and public-health impact of the P.1 (Gamma) variant in Brazil, supporting variant-specific interpretations

16. Mlcochova P, Kemp SA, Dhar MS, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*. 2021;599(7883):114-9.

17. Bastos LS, Niquini RP, Lana RM, et al. COVID-19 and hospitalizations for SARI in Brazil: a comparison up to the 12th epidemiological week of 2020. *Cad Saude Publica*. 2020;36(4):e00070120.

18. Silva ADD, Veiga ABGD, Cruz OG, et al. Severe acute respiratory infection surveillance in Brazil: the role of public, private and philanthropic healthcare units. *Health Policy Plan*. 2022;37(9):1075-85.

19. Monteiro N. Laboratórios públicos ampliam em 869% capacidade de testagem para Covid-19 no Brasil [Internet]. Brasília (DF): Ministério da Saúde; 2020 [updated 2022 Nov 1; cited 2025 Jul 31]. Available from: https://www.gov.br/saude/pt-br/assuntos/noticias/2020/julho/laboratorios-publicos-ampliam-em-869-capacidade-de-testagem-para-covid-19-no-brasil.

20. Eales O, Plank MJ, Cowling BJ, et al. Key challenges for respiratory virus surveillance while transitioning out of acute phase of COVID-19 pandemic. *Emerg Infect Dis*. 2024;30(2):e230768.

21. World Health Organization. Integrated sentinel surveillance of influenza and SARS-CoV-2 and the development of the Global Influenza Surveillance and Response System Plus: virtual meeting, 12–14 October 2021 [Internet]. Geneva: WHO; 2022 Jun 15 [cited 2025 Jul 30]. Available from: https://www.who.int/publications/i/item/9789240050228

22. Baker RE, Park SW, Yang W, et al. The impact of COVID-19 nonpharmaceutical interventions on the future dynamics of endemic infections. *Proc Natl Acad Sci U S A*. 2020;117(48):30547-53.

23. Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003-2004. *N Engl J Med*. 2005;353(24):2559-67.

24. Dee K, Goldfarb DM, Haney J, et al. Human rhinovirus infection blocks severe acute respiratory syndrome coronavirus 2 replication within the respiratory epithelium: implications for COVID-19 epidemiology. *J Infect Dis*. 2021;224(1])31-8.

25. Morelli T, Freeman A, Staples KJ, et al. Hidden in plain sight: the impact of human rhinovirus infection in adults. *Respir Res*. 2025;26(1):120.

26. Huang QS, Wood T, Jelley L, et al. Impact of the COVID-19 nonpharmaceutical interventions on influenza and other respiratory viral infections in New Zealand. *Nat Commun*. 2021;12(1):1001.

27. Maison N, Omony J, Rinderknecht S, et al. Old foes following new ways? Pandemic-related changes in the epidemiology of viral respiratory tract infections. *Infection*. 2024;52(1):209-18.

28. **World Health Organization. Implementing the integrated sentinel surveillance of influenza and other respiratory viruses. Geneva: WHO; 2024–2025. ISBN: 978-92-4-010143-2.
Key contribution: Establishes an authoritative operational framework for integrated sentinel surveillance of respiratory viruses, directly underpinning the surveillance rationale and structure adopted in the study.

29. *European Centre for Disease Prevention and Control (ECDC). European Respiratory Virus Surveillance Summary (ERVISS). Stockholm: ECDC; 2025.
Key contribution: Provides a contemporary example of standardized, integrated respiratory virus surveillance reporting, informing contextualization of indicators and interpretation of circulation patterns.

30. *Upadhyay P, Orozco M, Warren D, et al. Performance assessment of multiplex real-time PCR platforms for SARS-CoV-2/Flu A/Flu B/RSV detection. Diagnostics (Basel). 2024;14(8):857.

Key contribution: Supplies performance evidence supporting the feasibility and analytical utility of multiplex real-time PCR for concurrent detection of major respiratory viruses.

31. *Berry GJ, Hayden RT. Laboratory testing for respiratory viruses for the clinician. Cleve Clin J Med. 2024;91(9 Suppl 1):S42–S51.
Key contribution: Synthesizes clinically oriented guidance on respiratory virus diagnostics, reinforcing the interpretation and practical implications of laboratory confirmation beyond syndromic assessment.

32. Dhanasekaran V, Sullivan S, Edwards K, et al. Human seasonal influenza under COVID-19 and the potential consequences of prolonged suppression. Nat Commun. 2022;13:5378.

33. Abu-Raya B, Hamid JS, Papenburg J, et al. Why has the epidemiology of RSV changed during the COVID-19 pandemic? EClinicalMedicine. 2023;62:102153.

34. World Health Organization. Influenza Update No. 461 – integrated monitoring of influenza and SARS-CoV-2 (RespiMART). Geneva: WHO; 2023.

35. Secretaria de Estado de Saúde de Minas Gerais. Manual para o diagnóstico da covid-19: versão 7 [Internet]. Belo Horizonte (MG): Secretaria de Estado de Saúde de Minas Gerais; 2022 [cited 2025 Dec 30]. Available from: https://www.saude.mg.gov.br/wp-content/uploads/2025/02/05-01-Manual_para_o_Diagnostico_da_covid-19-versao_7_-_final.pdf

36. Secretaria de Estado de Saúde de Minas Gerais. Nota Informativa nº 1/2025 – Aumento da circulação de vírus respiratórios em Minas Gerais [Internet]. Belo Horizonte (MG): Secretaria de Estado de Saúde de Minas Gerais; 2025 [cited 2025 Dec 30]. Available from: https://www.saude.mg.gov.br/wp-content/uploads/2025/07/SEI_114744471_Nota_1.pdf

37. Martins RB, Rocha LP, Prates MM, et al. Respiratory DNA viruses are undetectable in nasopharyngeal secretions from adenotonsillectomized children. *PLoS One* [Internet]. 2017 [cited 2025 Jul 28];12[3]. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0174188

38. ARTIC Network. artic-ncov2019: ARTIC network novel coronavirus 2019 sequencing protocol [Internet]. GitHub; [cited 2025 Dec 30]. Available from: https://github.com/artic-network/artic-ncov2019

39. Aksamentov I, Roemer C, Hodcroft EB, et al. Nextclade: clade assignment, mutation calling and quality control for viral genomes. *J Open Source Softw* [Internet]. 2021 [cited 2025 Jul 28];6(67). Available from: https://joss.theoj.org/papers/10.21105/joss.03773

40. O'Toole Á, Scher E, Underwood A, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. *Virus Evol* [Internet]. 2021 [cited 2025 Jul 28];7(2). Available from: https://academic.oup.com/ve/article/7/2/veab064/6315289

41. Agência Nacional de Vigilância Sanitária (ANVISA). Nota Técnica GVIMS/GGTES/ANVISA nº 04/2020: orientações para serviços de saúde—medidas de prevenção e controle durante a assistência aos casos suspeitos ou confirmados de infecção pelo SARS-CoV-2. Brasília: ANVISA; 2024 Jun 24. [Internet]. Acesso em 21 out 2025. Disponível em: ANVISA.

42. Conselho Nacional de Secretários de Saúde (CONASS). Guia orientador para enfrentamento da COVID-19 nas Redes de Atenção à Saúde. 4ª ed. Brasília: CONASS; 2021. [Internet]. Acesso em 21 out 2025.

43. Centers for Disease Control and Prevention (CDC). Clinical Course: Progression, Management, and Treatment. Atlanta (GA): CDC; 2024 Jun 13. [Internet]. Acesso em 21 out 2025. Disponível em: CDC.

44. World Health Organization (WHO). Clinical management of COVID-19: interim guidance. Geneva: WHO; 2021 Jan 25. [Internet]. Acesso em 21 out 2025. Disponível em: WHO.

45. Jacoby P, Glass K, Moore HC. Characterizing the risk of respiratory syncytial virus in infants with older siblings: a population-based birth cohort study. *Epidemiol Infect*. 2017;145(2):266-71.

46. Piroth L, Cottenet J, Mariet AS, et al. Comparison of the characteristics, morbidity, and mortality of COVID-19 and seasonal influenza: a nationwide, population-based retrospective cohort study. *Lancet Respir Med*. 2021;9(3):251-9.

47. Wang B, Andraweera P, Elliott S, et al. Asymptomatic SARS-CoV-2 infection by age: a global systematic review and meta-analysis. *Pediatr Infect Dis J*. 2023;42(3):232-9.

48. Sah P, Fitzpatrick MC, Zimmer CF, et al. Asymptomatic SARS-CoV-2 infection: a systematic review and meta-analysis. *Proc Natl Acad Sci U S A*. 2021;118(34):e2109229118.

49. Boehmer TK, DeVies J, Caruso E, et al. Changing age distribution of the COVID-19 pandemic - United States, May-August 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(39):1404-9.

50. Centers for Disease Control and Prevention. COVID data tracker [homepage on the Internet]. Atlanta: U.S. Department of Health and Human Services, CDC; 2025 [cited 2025 Aug 1]. Available from: https://www.cdc.gov/covid-data-tracker/index.html#demographicsovertime

51. Monod M, Blenkinsop A, Xi X, et al. Age groups that sustain resurging COVID-19 epidemics in the United States. *Science* [Internet]. 2021 [cited 2025 Jul 28];371(6536). Available from: https://www.science.org/doi/10.1126/science.abe8372

52. Ye W, Lu W, Tang Y, et al. Identification of COVID-19 clinical phenotypes by principal component analysis-based cluster analysis. *Front Med [Lausanne]* [Internet]. 2020 [cited 2025 Jul 30];7. Available from: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2020.570614/full

53. Epsi NJ, Chenoweth JG, Blair PW, et al. Precision symptom phenotyping identifies early clinical and proteomic predictors of distinct COVID-19 sequelae. *J Infect Dis*. 2024;232[1]:39-49.

54. Geismar C, Nguyen V, Fragaszy E, et al. Symptom profiles of community cases infected by influenza, RSV, rhinovirus, seasonal coronavirus, and SARS-CoV-2 variants of concern. *Sci Rep* [Internet]. 2023 [cited 2025 Jul 30];13(1). Available from: https://www.nature.com/articles/s41598-023-38869-1

55. Dietz E, Pritchard E, Pouwels K, et al. SARS-CoV-2, influenza A/B and respiratory syncytial virus positivity and association with influenza-like illness and self-reported symptoms, over the 2022/23 winter season in the UK: a longitudinal surveillance cohort. *BMC Med*. 2024;22(1):143.

56. Rao S, Armistead I, Tyler A, Lensing M, Dominguez SR, Alden NB. Respiratory syncytial virus, influenza, and coronavirus disease 2019 hospitalizations in children in Colorado during the 2021-2022 respiratory virus season. *J Pediatr*. 2023;260:113491.

57. Orellana JDY, Cunha GM, Marrero L, et al. In-hospital case fatality ratio due to COVID-19 in scenarios with and without circulation of the Gamma variant (Brazil). Rev Bras Epidemiol. 2021;30(4):e2021709.

58. Murari TB, Behar PRP, et al. Retrospective cohort of hospitalized COVID-19 patients across Omicron, Gamma and Delta periods in Brazil. Viruses. 2022;14(9):1886.

59. Banho CA, Sacchetto L, Campos GRF, et al. Impact of SARS-CoV-2 Gamma lineage introduction and COVID-19 vaccination on the epidemiological landscape of a Brazilian city. *Commun Med [Lond]*. 2022;2:41.

60. Freitas ARR, Beckedorff OA, Cavalcanti LPG, et al. The emergence of novel SARS-CoV-2 variant P.1 in Amazonas [Brazil] was temporally associated with a change in the age and sex profile of COVID-19 mortality: a population based ecological study. *Lancet Reg Health Am*. 2021;1:100021.

61. Naveca FG, Nascimento V, de Souza VC, et al. COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat Med*. 2021;27(7):1230-8.

62. Slavov SN, de La-Roque DGL, da Costa PNM, et al. Dynamics of SARS-CoV-2 variants of concern in vaccination model city in the state of Sao Paulo, Brazil. *Viruses*. 2022;14(10):2148.

63. Giovanetti M, Fonseca V, Wilkinson E, et al. Replacement of the Gamma by the Delta variant in Brazil: impact of lineage displacement on the ongoing pandemic. *Virus Evol*. 2022;8(1):veac024.

64. Silva JP, de Lima AB, Alvim LB, et al. Delta variant of SARS-CoV-2 replacement in Brazil: a national epidemiologic surveillance program. *Viruses*. 2022;14(5):847.

65. Petrone ME, Lucas C, Menasche B, et al. Insights into the limited global spread of the immune evasive SARS-CoV-2 variant Mu. *medRxiv* [Preprint]. 2022:2022.03.28.22273077. Available from: https://doi.org/10.1101/2022.03.28.22273077

66. Markov PV, Ghafari M, Beer M, et al. The evolution of SARS-CoV-2. *Nat Rev Microbiol*. 2023;21(6):361-79.

67. Giovanetti M, Fonseca V, Wilkinson E, et al. Replacement of the Gamma by the Delta variant in Brazil: Impact of lineage displacement on the ongoing pandemic. Virus Evol. 2022;8(1):veac024. Published 2022 Mar 18. doi:10.1093/ve/veac024

68. Krumbein H, Kümmel LS, Fragkou PC, et al. Respiratory viral co-infections in patients with COVID-19 and associated outcomes: a systematic review and meta-analysis. *Rev Med Virol*. 2023;33(1):e2365.

69. Georgakopoulou VE. Insights from respiratory virus co-infections. *World J Virol*. 2024;13[4]:98600.

70. Alosaimi B, Naeem A, Hamed ME, et al. Influenza co-infection associated with severity and mortality in COVID-19 patients. *Virol J*. 2021;18(1):127.

71. Scotta MC, Chakr VC, de Moura A, et al. Respiratory viral coinfection and disease severity in children: a systematic review and meta-analysis. *J Clin Virol*. 2016;80:45-56.

72. Goldberg EM, Hasegawa K, Lawrence A, et al. Viral coinfection is associated with improved outcomes in emergency department patients with SARS-CoV-2. *West J Emerg Med*. 2021;22(6):1262-9.

73. Ahmed, R. COVID-19 and Co-Infections: A Review of Clinical Implications and Management Challenges. Premier Journal of Science. 2025.https://doi.org/10.70389/pjs.100091

74. Drucker DJ. Diabetes, obesity, metabolism, and SARS-CoV-2 infection: the end of the beginning. *Cell Metab*. 2021;33(3):479-98.

75. Popkin BM, Du S, Green WD, et al. Individuals with obesity and COVID-19: a global perspective on the epidemiology and biological relationships. *Obes Rev* [Internet]. 2020 [cited 2025 Jul 28];21(11). Available from: https://onlinelibrary.wiley.com/doi/10.1111/obr.13128

76. Pei L, Chen Y, Zheng X, et al. Comorbidities prolonged viral shedding of patients infected with SARS-CoV-2 Omicron variant in Shanghai: a multi-center, retrospective, observational study. *J Infect Public Health*. 2023;16(2):182-9.

77. Hua S, Yang Y, Zou D, et al. COVID-19 and metabolic comorbidities: an update on emerging evidences for optimal therapies. *Biomed Pharmacother*. 2021;140:111685.

78. Bui, Dinh S, et al. Lung Function Levels Influence the Association between Obesity and Risk of COVID-19. American journal of respiratory and critical care medicine vol. 204,9 (2021): 1106-1108. doi:10.1164/rccm.202105-1100LE

79. Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 [COVID-19]. *JAMA Cardiol*. 2020;5(7):811-8.

80. Clerkin KJ, Fried JA, Raikhelkar J, et al. COVID-19 and cardiovascular disease. *Circulation*. 2020;141(20):1648-55.

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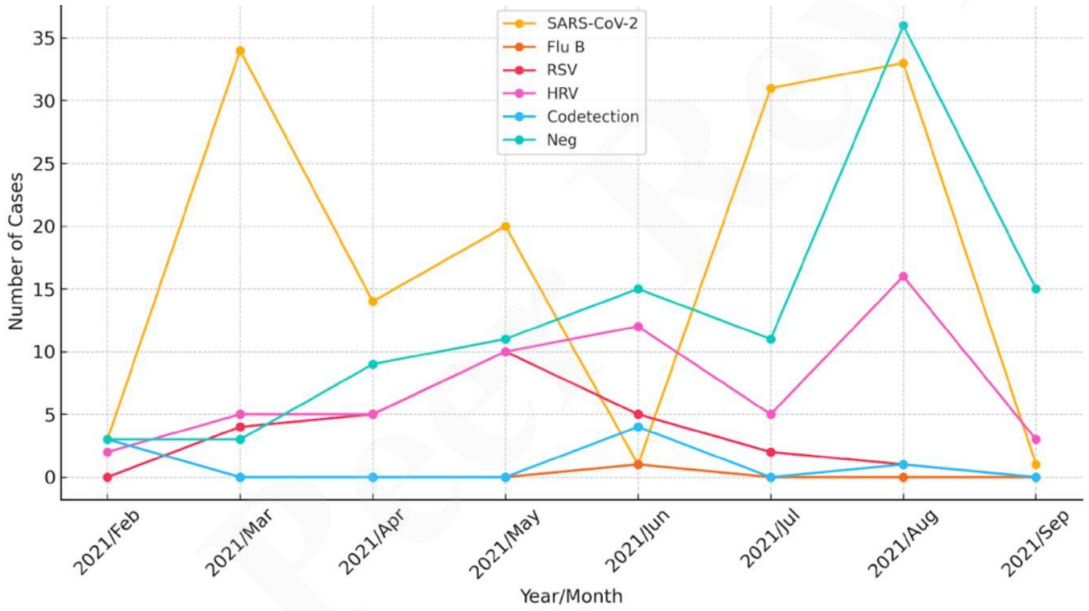
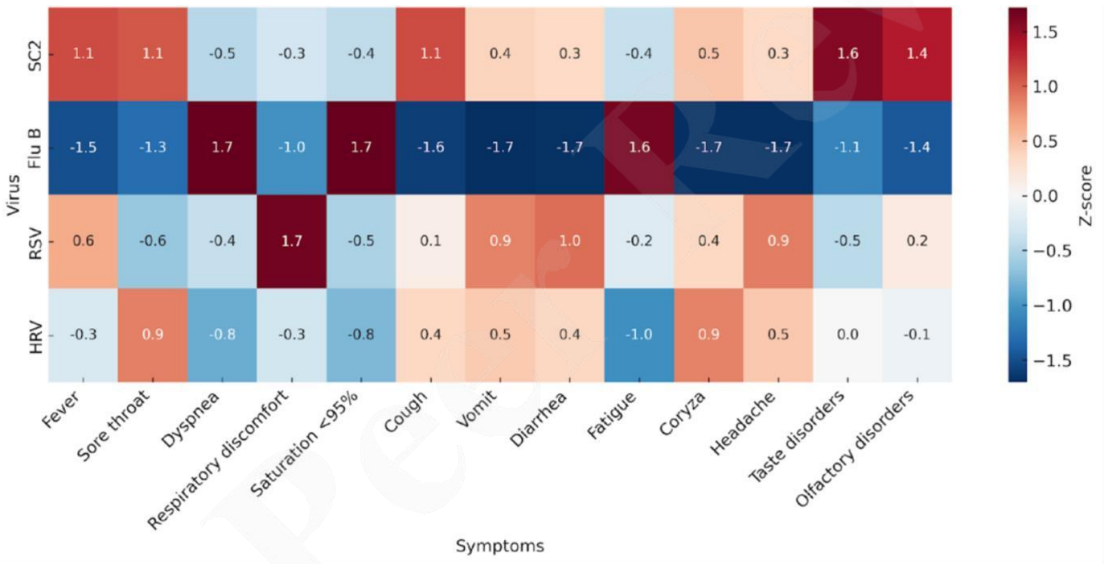
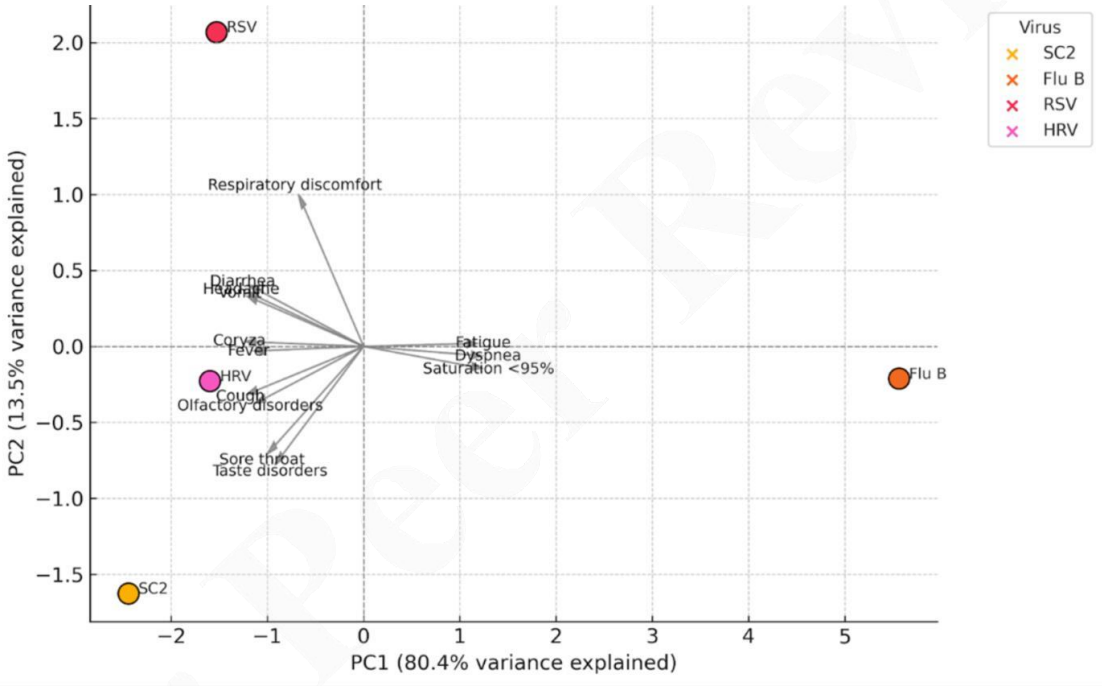
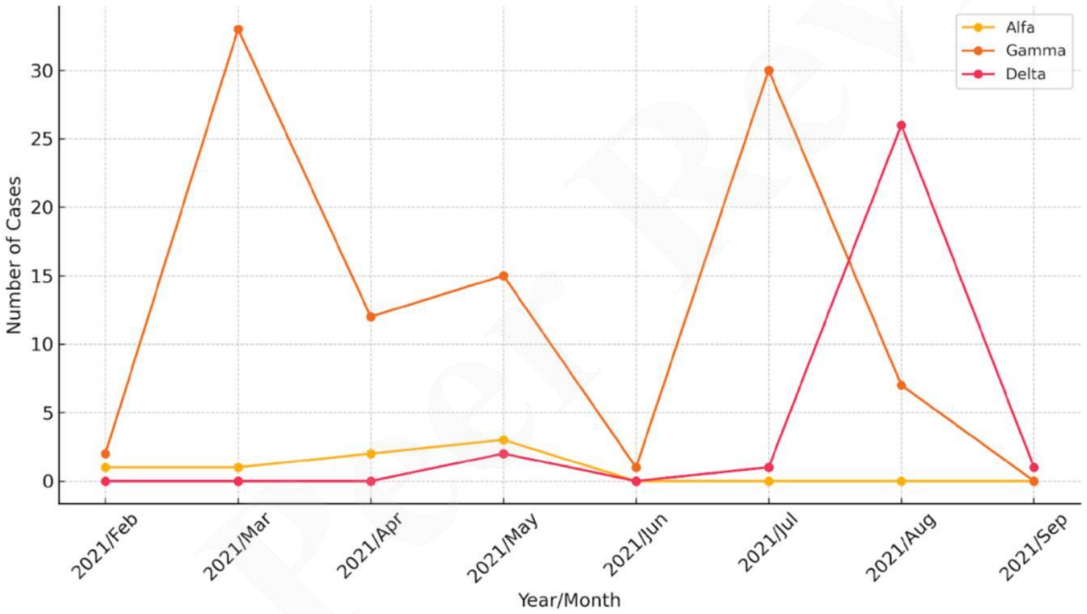
Supplementary Table 6. Association between FLU B infection and comorbidities. Comparison between patients with FLU B monoinfection and test-negative controls (NEG). For each comorbidity, the crude odds ratio reflects the relative odds of having the virus among patients with versus without the comorbidity.









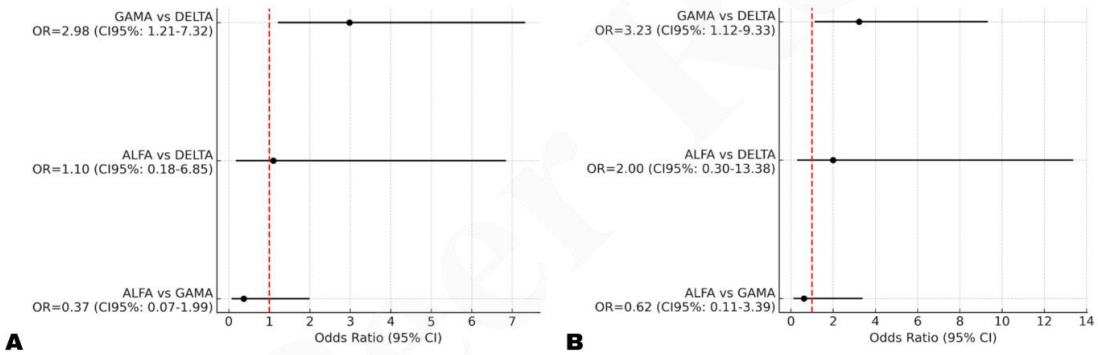


Table 5. Association between HRV infection and comorbidities. Comparison between patients with HRV monoinfection and test-negative controls (NEG). For each comorbidity, the crude odds ratio reflects the relative odds of having the virus among patients with versus without the comorbidity.

Comorbidity	N with data	With comorbidity, n (%)	HRV among with, %	HRV among without, %	OR (95% CI)	p-value
Hypertension	112	20 (17.9)	25.0	39.1	0.52 (0.17–1.55)	0.309
Chronic respiratory disease	112	5 (4.5)	20.0	37.4	0.42 (0.05–3.88)	0.651
Diabetes	113	24 (21.2)	41.7	34.8	1.34 (0.53–3.36)	0.634
Obesity	113	2 (1.8)	100.0	36.0	NA	0.136
Chronic kidney disease	113	5 (4.4)	20.0	38.0	0.41 (0.04–3.78)	0.649
Immunosuppression	112	13 (11.6)	23.1	38.4	0.48 (0.12–1.86)	0.367
Genetic disease	113	0 (0.0)	NA	37.2	NA	1.000
Pregnancy	113	5 (4.4)	20.0	38.0	0.41 (0.04–3.78)	0.649
Neurological disease	113	3 (2.7)	33.3	37.3	0.84 (0.07–9.57)	1.000
Asthma	113	8 (7.1)	62.5	35.2	3.06 (0.69–13.54)	0.145
Cardiac disease	113	27 (23.9)	33.3	38.4	0.80 (0.32–2.00)	0.820
Postpartum	109	0 (0.0)	NA	36.7	NA	1.000

Table 4. Outcomes modeled as SARS-CoV-2 (any), Gamma, and Delta versus test-negative controls. Values are odds ratios (OR) with 95% confidence intervals and p-values. Multivariable models here include mutual adjustment among comorbidities (diabetes, obesity, cardiac disease) based on the available worksheet; demographics and vaccination were not present. Replace with fully adjusted estimates if/when those covariates are added.

Outcome	Predictor	N (model)	Cases (outcome)		OR (95% CI); p — Univariate	OR (95% CI); p — Multivariable	Covariates_adjusted	Notes
SARS-CoV-2 (any)	Cardiac disease	206.0	135.0		1.42 (0.75–2.71); p=0.282	1.30 (0.66–2.56); p=0.440	Diabetes + Obesity + Cardiac disease (mutual adjustment)	Demographic/vaccination covariates not available in this sheet
SARS-CoV-2 (any)	Diabetes	206.0	135.0		1.34 (0.66–2.71); p=0.413	1.24 (0.58–2.63); p=0.582	Diabetes + Obesity + Cardiac disease (mutual adjustment)	Demographic/vaccination covariates not available in this sheet
SARS-CoV-2 (any)	Obesity	206.0	135.0		10.14 (0.58–177.57); p=0.051	2566892854.78 (0.00–inf); p=0.999	Diabetes + Obesity + Cardiac disease (mutual adjustment)	Demographic/vaccination covariates not available in this sheet
Gamma (SARS-CoV-2)	Cardiac disease	170.0	99.0		2.00 (1.02–3.90); p=0.041			
Gamma (SARS-CoV-2)	Diabetes	171.0	99.0		1.33 (0.63–2.79); p=0.456			
Gamma (SARS-CoV-2)	—	170.0	99.0			—	—	
Gamma (SARS-CoV-2)	Obesity	170.0	99.0		9.16 (0.50–166.74); p=0.071			
Delta (SARS-CoV-2)	Cardiac disease	100.0	29.0		0.47 (0.14–1.54); p=0.205	0.39 (0.10–1.47); p=0.163	Diabetes + Obesity + Cardiac disease (mutual adjustment)	Demographic/vaccination covariates not available in this sheet
Delta (SARS-CoV-2)	Diabetes	100.0	29.0		1.08 (0.37–3.16); p=0.887	1.91 (0.55–6.61); p=0.306	Diabetes + Obesity + Cardiac disease (mutual adjustment)	Demographic/vaccination covariates not available in this sheet
Delta (SARS-CoV-2)	Obesity	100.0	29.0		10.52 (0.46–240.72); p=0.071	160434495.68 (0.00–inf); p=0.997	Diabetes + Obesity + Cardiac disease (mutual adjustment)	Demographic/vaccination covariates not available in this sheet

Table 6. Association between FLU B infection and comorbidities. Comparsion between patients with FLU B monoinfection and test-negative controls (NEG). For each comorbidity, the crude odds ratio reflects the relative odds of having the virus among patients with versus without the comorbidity.

Comorbidity	N with data	With comorbidity, n (%)	FLU B among with, %	FLU B among without, %	OR (95% CI)	p-value
Hypertension	74	15 (20.3)	0.0	5.1	0.00 (0.00–0.00)	1.000
Chronic respiratory disease	74	4 (5.4)	0.0	4.3	0.00 (0.00–0.00)	1.000
Diabetes	75	15 (20.0)	6.7	3.3	2.07 (0.18–24.50)	0.493
Obesity	74	0 (0.0)	NA	4.1	NA	1.000
Chronic kidney disease	74	4 (5.4)	0.0	4.3	0.00 (0.00–0.00)	1.000
Immunosuppression	74	11 (14.9)	9.1	3.2	3.05 (0.25–36.86)	0.387
Genetic disease	74	0 (0.0)	NA	4.1	NA	1.000
Pregnancy	74	4 (5.4)	0.0	4.3	0.00 (0.00–0.00)	1.000
Neurological disease	74	2 (2.7)	0.0	4.2	0.00 (0.00–0.00)	1.000
Asthma	74	3 (4.1)	0.0	4.2	0.00 (0.00–0.00)	1.000
Cardiac disease	74	19 (25.7)	5.3	3.6	1.47 (0.13–17.22)	1.000
Postpartum	72	0 (0.0)	NA	4.2	NA	1.000

Table 1. Demographic Data of Patients Included in the Study

	General	Positive (%)	Negative (%)	SC2 (%)	Flu B (%)	RSV (%)	HRV (%)	Coinfection (%)
Female	180 (53.4)	128 (54.7)	52 (50.5)	77 (56.2)	2 (50.0)	12 (44.4)	31 (53.4)	6 (75.0)
Male	157 (46.6)	106 (45.3)	51 (49.5)	60 (43.8)	2 (50.0)	15 (55.6)	27 (46.6)	2 (25.0)
Total	337 (100)	234 (100)	103 (100)	137 (100)	4 (100)	27 (100)	58 (100)	8 (100)
Age								
Mean	48.3	47.4	50.4	55.8	56.3	25.8	40.7	20.0
Median	51.0	51.5	51	59.0	64.5	4.0	40.5	22.5
SD	24.4	24.7	23.8	17.1	20.4	30.7	27.4	17.5
P		0.2998		<0.001				

SARS-CoV-2 (SC2), influenza B (FluB), respiratory syncytial virus (RSV), human rhinovirus (HRV), Standard Deviation (SD) and p-value (P)

Table 2. Risk factors for SARS-CoV-2 infection, aggregate and by variant.

	SARS-CoV-2		Gamma (SARS-CoV-2)		Delta (SARS-CoV-2)	
Predictor	Univariate OR (95% CI); p	Multivariable OR (95% CI); p	Univariate OR (95% CI); p	Multivariable OR (95% CI); p	Univariate OR (95% CI); p	Multivariable OR (95% CI); p
Diabetes	1.34 (0.66–2.71); p=0.413	1.24 (0.58–2.63); p=0.582	1.33 (0.63–2.79); p=0.456	—	1.08 (0.37–3.16); p=0.887	1.91 (0.55–6.61); p=0.306
Obesity	10.14 (0.58–177.57); p=0.051	—	9.16 (0.50–166.74); p=0.071	—	10.52 (0.46–240.72); p=0.071	—
Cardiac disease	1.42 (0.75–2.71); p=0.282	1.30 (0.66–2.56); p=0.440	2.00 (1.02–3.90); p=0.041	—	0.47 (0.14–1.54); p=0.205	0.39 (0.10–1.47); p=0.163

Univariate = single predictor; multivariable = mutual adjustment among diabetes, obesity, cardiac disease. Full adjustment (age, sex, vaccination, calendar period, region) will be added when available. Software: Python 3.11.8 (pandas 1.5.3; NumPy 1.24.0; statsmodels 0.13.5). Cells marked ("—") indicate non-convergence or instability due to sparse data (quasi-separation); in these instances, standard maximum-likelihood logistic regression is unreliable. If desired, penalized logistic regression (e.g., Firth or L2 ridge) can be used to obtain finite, more stable estimates.

Table 3. Presents the crude association between detection of any respiratory virus (including coinfections) and underlying comorbidities. The outcome was defined as infected (any virus detected) versus non-infected (NEG).

Comorbidity	N with data	With comorbidity, n (%)	Infected among with, %	Infected among without, %	OR (95% CI)	p-value
Hypertension	276	45 (16.3)	66.7	75.8	0.64 (0.32–1.27)	0.263
Chronic respiratory disease	276	13 (4.7)	69.2	74.5	0.77 (0.23–2.58)	0.746
Diabetes	277	59 (21.3)	76.3	73.4	1.17 (0.60–2.28)	0.739
Obesity	277	11 (4.0)	100.0	73.3	inf (inf–inf)	0.071
Chronic kidney disease	277	11 (4.0)	63.6	74.8	0.59 (0.17–2.08)	0.481
Immunosuppression*	276	19 (6.9)	47.4	76.3	0.28 (0.11–0.72)	0.011
Genetic disease	277	0 (0.0)	NA	74.4	NA	1.000
Pregnancy**	276	5 (1.8)	20.0	75.3	0.08 (0.01–0.75)	0.016
Neurological disease	277	11 (4.0)	81.8	74.1	1.58 (0.33–7.47)	0.735
Asthma	224	15 (6.7)	80.0	67.5	1.93 (0.53–7.06)	0.399
Cardiac disease	277	74 (26.7)	75.7	73.9	1.10 (0.59–2.04)	0.877
Postpartum	273	0 (0.0)	NA	74.7	NA	1.000

**Asterisk indicates statistically significant associations ($p < 0.05$), including both risk-increasing and potentially protective patterns.*

Interpretation of significant associations:

Immunosuppression*

In this cohort, 19 out of 276 patients (6.9%) were classified as immunosuppressed. Among immunosuppressed patients, 47.4% tested positive for a respiratory virus, whereas 76.3% of non-immunosuppressed patients had a positive result. The crude odds ratio was 0.28 (95% CI: 0.11–0.72; p = 0.011), indicating lower odds of virus detection among immunosuppressed individuals.

This apparent “protective” effect is unlikely to reflect a true biological benefit. Rather, it probably arises from differences in clinical pathways and testing indications. Immunosuppressed patients are often tested more frequently and at a lower threshold of clinical suspicion, which can increase the proportion of test-negative episodes captured in this group. As a result, immunosuppression should be interpreted here as a marker of differential selection and surveillance, not as a factor that reduces susceptibility to infection.

Pregnancy**

Only 5 out of 276 women with available data (1.8%) were pregnant. Among pregnant women, 20.0% tested positive for a respiratory virus, compared with 75.3% among non-pregnant women. The crude odds ratio was 0.08 (95% CI: 0.01–0.75; p = 0.016), suggesting markedly lower odds of infection in pregnant patients.

However, this finding must be interpreted with considerable caution. The sample size is extremely small, yielding an unstable estimate and wide confidence intervals. In addition, pregnant women may follow distinct care pathways and testing criteria (for example, routine screening or testing prompted by lower clinical thresholds), which can lead to an over-representation of test-negative results. Therefore, pregnancy is better viewed as a signal of different patterns of care and surveillance rather than genuine protection against respiratory virus infection in this dataset.

CAPÍTULO 2:

EPIDEMIOLOGICAL AND CLINICAL PATTERNS OF VIRAL RESPIRATORY INFECTIONS IN TWO REGIONS OF MINAS GERAIS, BRAZIL: IMPACT IN THE POST_PANDEMIC PERIOD

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Future Virology

Epidemiological and Clinical Patterns of Viral Respiratory Infections in Two Regions of Minas Gerais, Brazil: Impact in the Post-Pandemic Period

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Authors	Katia Ozanic, Mateus Cunha de Oliveira Galdino, Leticia Hagale de Queiroz, Vanessa Cordeiro Dias, Vania Lucia da Silva, Alessandra Barbosa Ferreira Machado, Aripuana Watanabe, Claudio Galuppo Diniz

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Title: Epidemiological and Clinical Patterns of Viral Respiratory Infections in Two Regions of Minas Gerais, Brazil: Impact in the Post-Pandemic Period

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Abstract

Background: When multiple respiratory viruses co-circulate after the COVID-19 pandemic, interpreting severe acute respiratory infection (SARI) trends requires simultaneous assessment of pathogen prevalence, age distribution and severity markers. Methods: We retrospectively evaluated 264 suspected SARI cases (June 2023-September 2024) from two health regions in Minas Gerais, Brazil, tested by RT-qPCR for SARS-CoV-2, influenza A/B (Flu A/B), respiratory syncytial virus (RSV) and human rhinovirus (HRV). Results: RSV had the highest detection rate (14.77%), predominated in infants, and yielded the highest hospitalization (69.23%) and ICU admission (55.55%) rates. HRV (11.36%) circulated year-round, whereas SARS-CoV-2 (7.95%) showed irregular activity. Influenza A/B (9.46%) and HRV detections were concentrated among older adults and were also associated with severe presentations. Principal component analysis (PCA) suggested pathogen-specific symptom signatures, and only HRV viral load correlated with hospitalization (p=0.048). We contextualize these observations with contemporary post-pandemic evidence identified through targeted database searches. Conclusions: SARI surveillance should shift toward integrated, year-round monitoring with consistent clinical metadata capture. Prevention strategies, particularly influenza vaccination and RSV immunization, are likely to deliver the most immediate reductions in severe disease burden.

Keywords: Viral respiratory infections; SARS-CoV-2; Respiratory Syncytial Virus; Influenza; Human rhinovirus (HRV); Severe Acute Respiratory Syndrome; Epidemiological surveillance; Post-pandemic period; qPCR; Differential diagnosis of viral respiratory infections.

1. Introduction

Severe acute respiratory infection (SARI) is a severe respiratory condition characterized by symptoms such as fever, cough, and respiratory distress, which can progress to respiratory failure and the need for ventilatory support [1,2]. SARI represents a significant cause of morbidity and mortality worldwide, primarily associated with infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza viruses A and B (Flu A/B), human rhinovirus (HRV) and respiratory syncytial virus (RSV), all of which exhibit high transmissibility and a substantial impact on healthcare systems [3-5]. Effective diagnosis and clinical management of these pathogens are essential to reducing complications and preventing severe outcomes [6].

Despite the predominance of these three viruses, other viral etiologies can also trigger SARI, including, adenoviruses, parainfluenza, and metapneumovirus, highlighting the need for precise differential diagnosis, as the clinical symptoms of these infections are similar, making etiological identification challenging without specific laboratory tests [7].

Molecular biology testing, particularly RT-qPCR, is currently the gold standard for SARI diagnosis due to its high sensitivity and specificity in detecting low viral loads [8]. The COVID-19 pandemic has affected the circulation of other respiratory viruses and altered seasonal patterns, leading to an increased occurrence of coinfections and greater variability in clinical presentations [9].

Although SARI has been extensively studied in relation to SARS-CoV-2, Influenza, HRV and RSV, there remains a knowledge gap regarding the relative contribution of other respiratory viruses to severe cases after the pandemics. The lack of detailed

epidemiological and clinical data on these infections, after the international health emergency, hinders the implementation of effective surveillance and response strategies. The post pandemic scenario underscores the need for studies that systematically investigate the role of different pathogens in SARI occurrence, particularly in settings with high viral circulation and frequent coinfections [10,11].

The present study aimed to evaluate the clinical and epidemiological aspects of patients diagnosed with SARI, in a post pandemic moment, from two Regional Health Management Offices in the state of Minas Gerais, Brazil. The primary focus was on identifying the main etiological agents, including RSV, Influenza, HRV and SARS-CoV-2. The underlying hypothesis is that, although SARI is classically associated with SARS-CoV-2, Influenza, HRV and RSV, it may also be triggered by other pathogens, whose detection and characterization could contribute to improving the diagnosis and clinical management of such cases.

2. Objectives

The present study aimed to evaluate the clinical and epidemiological characteristics of patients diagnosed with SARI during the post-pandemic period in two Regional Health Management Offices of the Minas Gerais State Health Department , Barbacena and Leopoldina, covering 48 municipalities in Minas Gerais, Brazil. Specifically, we sought to identify the main etiological agents routinely investigated in this setting, including RSV, influenza viruses, HRV, and SARS-CoV-2, and to characterize SARI cases that remained negative for these pathogens, thereby supporting the inference that other respiratory viruses and/or pathogens may be involved. We hypothesized that, beyond the classical agents, a substantial proportion of SARI may be attributable

to additional respiratory pathogens, and that recognizing this etiological gap is relevant to strengthening diagnostic strategies and informing clinical management and surveillance.

3. Materials and Methods

This is a retrospective cross-sectional study in which 264 samples of nasopharyngeal swabs, bronchoalveolar lavage or tracheal aspirate were collected between June 19, 2023 and September 15, 2024 from patients with suspected SARI. The samples were collected in hospitals, intensive care units, and Basic Health Units, from two GRS of SES: GRS of Barbacena and GRS of Leopoldina. Clinical and epidemiological data were collected, including sex, age, symptoms, comorbidities, education, area of residence (urban/rural), hospitalization and laboratory results.

3.1. Ethical Approval

Ethical approval was obtained from the Human Research Ethics Committee (protocol no. 710133723.3.0000.5147). Although complete anonymity was not feasible because a small number of personal identifiers was required for data collection and verification, all procedures were conducted under strict confidentiality. Prior to analysis, all datasets were de-identified and each record was assigned a unique study code. The file linking study codes to individual identities was stored separately from the analytical database and was accessible only to the principal investigator and specifically authorized personnel. Electronic data were maintained on secure institutional computers/servers with password protection and access restrictions, with regular backups. Sensitive files were further safeguarded through restricted folder permissions and, where available, encryption. No identifiable information will be

disclosed in any publication derived from this study. The local ethics committee granted a waiver of informed consent because the study was conducted as a diagnostic action offered to the population covered by two GRS of the State Health Department, GRS of Barbacena and GRS of Leopoldina, and required anonymization of personal data by the study coordinators.

3.2. Laboratory Testing

Viral RNA extraction was performed using the KingFisher Flex extraction (ThermoFisher, USA) according to the manufacturer's instructions.

The detection of viral RNA of SARS-CoV-2, Flu A/B and RSV was performed using the commercial TaqMan™ SARS-CoV-2, Flu A/B, RSV Multiplex Assay kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. Detection of viral RNA for Influenza A and Influenza B was performed using the IBMP Biomol Flu A, Flu B and COVID kit (IBMP, Brazil), according to the manufacturer's instructions. Samples tested for SARS-CoV-2, Flu A/B, and RSV were subsequently screened for HRV using a SYBR Green-based RT-qPCR assay. Primers targeting the 5' untranslated region of the HRV genome, as described by Martins et al. (2017) [12].

3.3 Statistical analysis and data treatment

Descriptive statistics were used to characterize the dataset, with categorical variables expressed as frequencies and continuous variables summarized by central tendency and dispersion. Associations were tested using Chi-square or Fisher's exact test, while group comparisons employed Student's t-test or one-way ANOVA, adopting a 5% significance level. Results were reported as odds ratios with 95% confidence intervals, using SPSS v20. To assess symptom distribution across viral etiologies, Principal Component Analysis (PCA) was applied to 12 standardized clinical symptoms in five infection groups. Components with eigenvalues >1 were retained, with the first three

explaining over 90% of the variance. Analyses and visualizations (biplots, scree plots, heatmaps) were performed in Python (pandas, matplotlib, seaborn, scikit-learn)..

4. Results

A total of 264 samples from patients suspected of Severe Acute Respiratory Syndrome (SARS) were analyzed. These samples were collected from hospitals, Intensive Care Units (ICUs), and Primary Health Care Units (PHCUs). The collection spanned 36 municipalities within GRS of Barbacena and Leopoldina, in the state of Minas Gerais, Brazil. The distribution of patients by residential area showed that 90.77% lived in urban areas and 9.23% in rural areas.

The average age of the patients was 31,2 years (SD = 33.7), with a median of 14.0 years. The sex distribution showed 54.33% male and 45.67% female.

SARS-CoV-2 was detected in 21 patients (7.95%), RSV in 39 patients (14.77%), and Influenza A/B in 25 patients (9.46%), with Influenza A representing the predominant type. HRV was identified in 30 patients (11.36%). The remaining 142 patients (53.78%) tested negative for all viruses included in the assay and were classified as Severe Acute Respiratory Infections of other etiologies.

A total of seven cases of viral coinfection were identified, corresponding to 2.65% of the study population. The following combinations were observed: HRV and SARS-CoV-2 in one sample; SARS-CoV-2 and RSV in one sample; HRV and Influenza A in one sample; HRV and RSV in five samples.

The temporal distribution and seasonality of positive cases for SARS-CoV-2, RSV, Influenza A/B, and HRV, as well as cases classified as SARI of undefined etiology, are presented in Figure 1. Among the detected pathogens, RSV was the most frequently identified virus, with 39 positive cases. A clear seasonal pattern was observed, with a significant increase in detection from March to June 2024, peaking in May. Influenza A was identified in 25 cases, with circulation beginning in January 2024 and gradually increasing until reaching a peak in August (11 cases). In contrast, Influenza B was detected in only one sample, in September 2024. SARS-CoV-2 was detected in 21 samples, showing a sporadic and discontinuous circulation pattern, primarily in September and October 2023, and again in February and September 2024. A reduction in SARS-CoV-2 detection was observed during months of high RSV

activity. HRV was detected in 30 samples and presented continuous circulation over several months, especially between October 2023 and July 2024, with peaks in October 2023 and June–July 2024. Finally, cases without a defined viral etiology (SARI of other etiologies) were predominant in November and December 2023 and July-August 2024, corresponding to periods of lower detection of the four main target viruses.

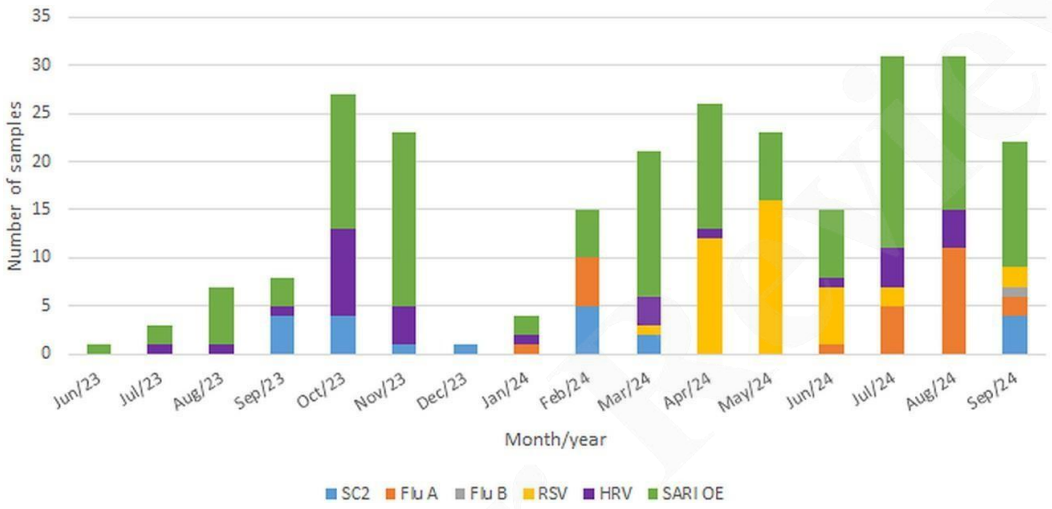

Figure 1: Monthly distribution of SARS-CoV-2, Flu A, Flu B, RSV, HRV, and SARI of undefined etiology cases during the study period. Temporal trends and potential viral interactions were analyzed.

The distribution of positive cases by sex varied among the different viral groups. Among the SARS-CoV-2 cases, a higher proportion of female patients was observed (59.1%), while RSV showed a predominance of male cases (55.8%). For Influenza A/B, the distribution was relatively balanced, with 52% of male cases and 48% of female cases. HRV showed the highest male predominance, with 62.2% of positive cases occurring in men and 37.8% in women. There was no statistically ignificant association between the type of virus and the sex of the infected individuals ($p > 0.05$).

Regarding age distribution, SARS-CoV-2 was predominantly detected in adults and elderly individuals, with a mean age of 57.4 years and a median of 69.5 years, indicating a higher burden in older populations. In contrast, RSV was most frequently detected in children, especially in early childhood, with a mean age of 6.8 years and a median of 0.6 years. Influenza A/B showed a more balanced age distribution, with a

mean of 48.3 years and a median of 54.5 years, affecting both younger and older adults. HRV presented the most heterogeneous age profile, with a mean age of 34.3 years and a median of 7 years, reflecting a broad distribution across age groups.

The mean ages among the four viral groups yielded a p-value < 0.001, confirming a statistically significant difference in age distribution between viruses. Specifically, SARS-CoV-2 and RSV showed the greatest age disparity, reinforcing their epidemiological targeting of distinct age populations. In contrast, a t-test comparing the average ages of patients with positive and negative viral results produced a p-value of 0.447, indicating no statistically significant difference in age between the infected and non-infected groups.

The geographic analysis of the positive cases revealed a marked concentration in urban areas, which accounted for 90.77% of all detected infections. In contrast, only 9.23% of the cases originated from rural areas.

PCA was conducted to explore symptom patterns across different etiological groups of acute respiratory infections. The first three principal components (PCs) were retained, explaining a cumulative 92.2% of the total variance: PC1 (49.0%), PC2 (23.0%), and PC3 (20.2%).

PC1 was predominantly associated with respiratory severity, marked by symptoms such as dyspnea, respiratory discomfort, and low oxygen saturation (SpO_2 <95%). PC2 captured systemic viral symptoms, including fever, loss of smell, and fatigue. PC3 reflected alternative or mild symptoms, particularly gastrointestinal manifestations such as vomiting, abdominal pain, and runny nose (Figure 2).

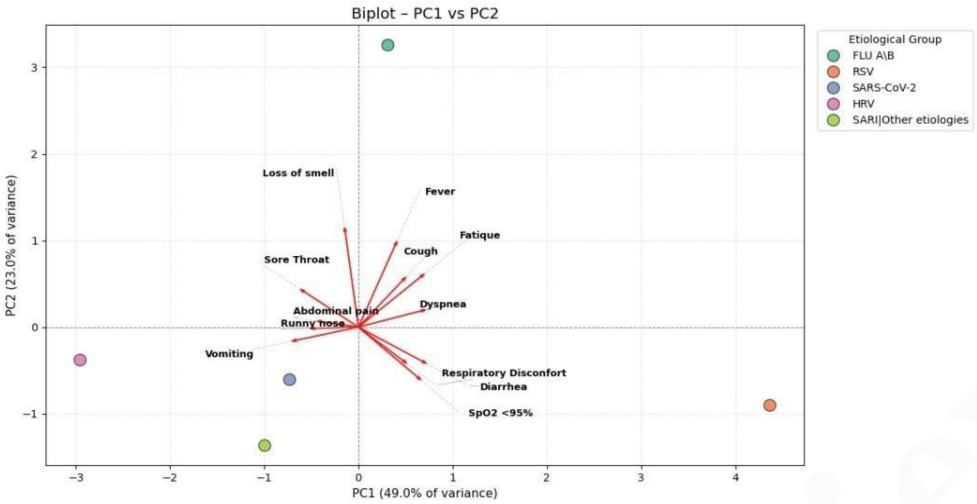

Figure 2: Biplot of Principal Component Analysis (PCA) showing the distribution of etiological groups along PC1 and PC2. PC1 represents respiratory severity, while PC2 reflects systemic viral symptom.

The biplots and correlation circles allowed the identification of distinct clinical profiles among the viral etiologies. RSV showed a strong positive association with PC1, confirming its link to severe respiratory illness. Influenza A/B was associated primarily with PC2, characterizing a typical viral syndrome. HRV scored high on PC3 and negatively on PC1, indicating a mild or extrapulmonary symptom pattern (Figure 3 and figure 4). SARS-CoV-2 and SARI/Other etiologies exhibited a central and diffuse position across the PCA space, with negative scores across all three components, suggesting clinical heterogeneity and lack of a dominant symptom profile (Figure 4). The 3D PCA plot, integrating all three principal components, provided a comprehensive view of the relative clinical distribution of etiologies, highlighting the polar contrast between RSV and HRV, and the intermediate or undefined presentation of SARS-CoV-2 and SARI (Figure 5).

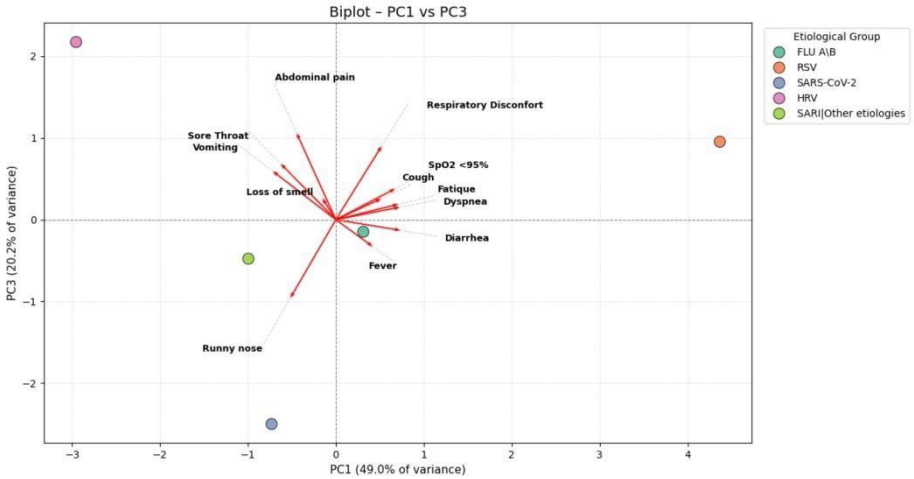

Figure 3: Biplot of PCA illustrating the distribution of etiological groups along PC1 and PC3. PC3 highlights gastrointestinal and mild symptomatology, contrasting with PC1 respiratory severity.

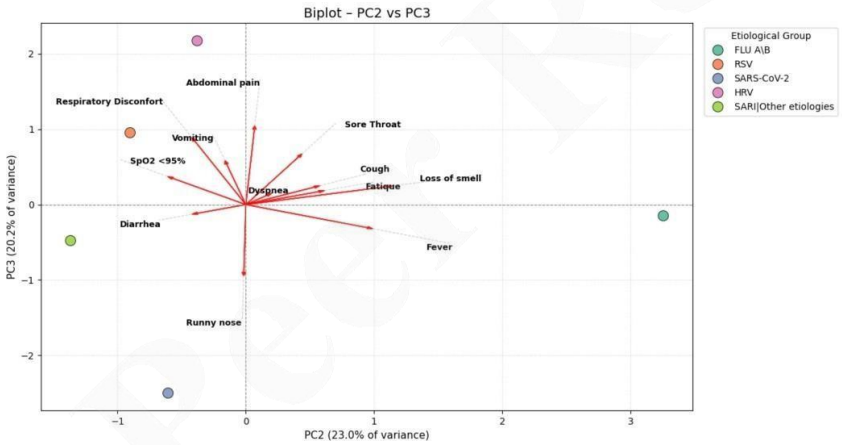

Figure 4: Biplot of PCA showing etiological group distribution along PC2 and PC3. This representation demonstrates overlapping and divergent clinical profiles among respiratory viroses.

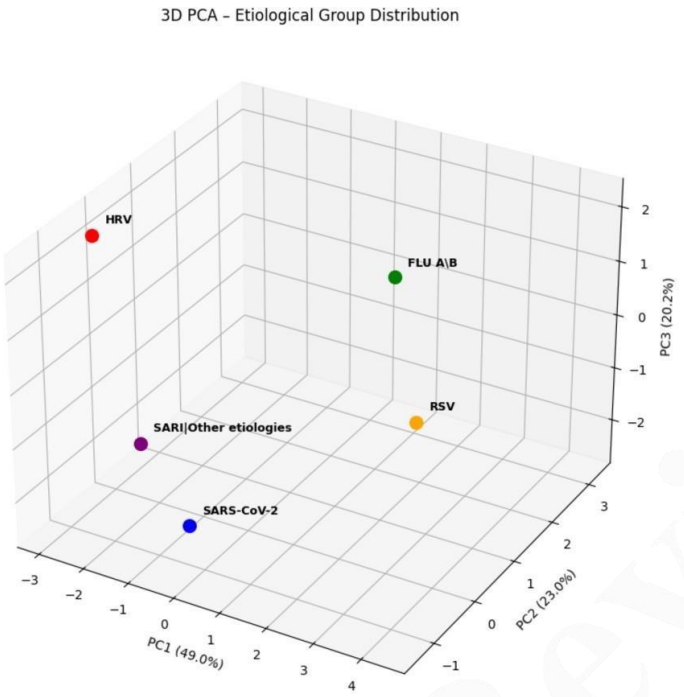

Figure 5: Three-dimensional PCA plot integrating PC1, PC2, and PC3. The 3D distribution highlights distinct syndromic patterns, including the contrast between RSV (severe respiratory illness) and HRV (milder or extrapulmonary presentation).

PCA can successfully differentiate syndromic profiles associated with specific respiratory viruses, although SARS-CoV-2 and undefined etiologies show significant overlap and variability.

Among SARS-CoV-2 positive cases (n = 21), 4 patients (19.04%) required hospitalization. For Influenza A/B (n = 25), 14 cases (56%) resulted in hospitalization. RSV-positive cases (n = 39) had the highest hospitalization rate, with 27 individuals (69.23%) admitted. HRV showed a hospitalization rate of 26.66% (8 out of 30). In the group categorized as “Other Severe Acute Respiratory Infections” (Other SARI; n = 142), 60 patients (42.25%) were hospitalized. The statistical analysis confirmed a significant association between viral etiology and hospitalization outcomes.

The highest ICU admission rates were observed among individuals infected with RSV (55.55%), SARS-CoV-2 (50%), and HRV (37.5%).

Among SARS-CoV-2 positive patients, 50% of hospitalizations required ICU care, especially in older adults and those with comorbidities. For RSV, 55.55% of

hospitalized cases were admitted to the ICU, predominantly children under 2 years of age (86%), with infants as the most affected group. In HRV infections, 37.5% of hospitalized patients needed ICU, all with underlying comorbidities, showing its severity in vulnerable hosts. For Influenza A/B, 21.42% of hospitalizations resulted in ICU admission, exclusively in patients aged ≥ 65 years, consistent with the known risk in the elderly. Among cases classified as Other Severe Acute Respiratory Infections (Other SARI) which were negative for the tested viruses 27.11% of hospitalizations progressed to ICU admission. The mean age of these ICU patients was 33.7 years, suggesting a more heterogeneous profile in this group.

The association between viral load and hospitalization was evaluated using Ct values. For SARS-CoV-2, mean Ct was 23.78 in hospitalized vs. 19.76 in non-hospitalized patients ($p = 0.41$). In Influenza A/B, Ct values were 28.10 vs. 25.66 ($p = 0.73$), and for RSV, 26.81 vs. 29.22 ($p=0.38$), with no significant differences. Only HRV showed significance: hospitalized patients had higher Ct values (30.09 vs. 25.31, $p=0.048$), indicating lower viral loads compared to non-hospitalized cases.

The analysis indicates that no consistent or significant correlation was found between Ct values and hospitalization for most viruses analyzed, with the exception of HRV, where the inverse association raises the hypothesis of host-related factors rather than viral load per se contributing to disease severity.

5. Discussion

The geographic distribution of cases revealed a predominance of urban origin compared to rural zones. Improved access to healthcare services and availability of diagnostic testing in urban areas may contribute to underreporting of respiratory infections in rural populations [13,14].

Etiological investigation using RT-qPCR detected SARS-CoV-2, RSV, HRV, and Influenza A/B, with Influenza A being the predominant subtype. These findings reflect the co-circulation of multiple respiratory viruses during the study period, corroborating reports in the literature that highlight seasonal overlap and viral outbreaks [15,16].

The high proportion of negative samples highlights a diagnostic gap, consistent with findings from the Instituto Todos pela Saúde (ITpS) expanded panel, which identified other pathogens such as metapneumovirus, adenovirus, parainfluenza, and atypical bactéria [16]. This underscores the need for broader molecular surveillance to improve

etiological characterization and guide public health actions. Viral co-infections occurred within the expected 2–10% range, and their exclusion from statistical analyses avoided confounding, consistent with recommendations from surveillance studies [17-19]. Future research with larger samples should assess the clinical impact of co-infections. RT-qPCR remains the gold standard for respiratory virus detection due to its high sensitivity and ability to identify infections at early stages [20,21].

The seasonal patterns of RSV and Influenza A/B were consistent with previous reports. RSV peaked in winter (June to August), a well-known pattern in Southern Hemisphere countries, and remains a major cause of pediatric hospitalizations in this period [22-24]. Influenza A/B showed two peaks, at the beginning of the year and in winter, influenced by meteorological factors such as temperature and humidity, with seasonality varying between temperate and tropical regions [25,26]. Influenza B was minimally detected, with only one case in September 2024, reflecting low circulation also reported in post-pandemic years [16].

SARS-CoV-2 showed irregular circulation without clear seasonality, indicating reduced dominance as the main SARI agent in the post-pandemic period, likely due to population immunity from prior infections or vaccination [27-30]. The decline in cases during RSV peaks suggests viral competition, supported by studies showing that SARS-CoV-2 proteins can inhibit RSV replication and trigger antiviral responses in co-infections [31]. Epidemiological data also confirm temporal displacement between these viruses, consistent with viral interference [32].

HRV maintains high community circulation throughout the year, with higher transmission during periods of environmental instability and school re-entry [33-35]**.**

The high proportion of cases without defined etiology, particularly in months with low detection of the four targeted viruses, highlights a significant diagnostic gap. These findings emphasize the importance of expanding the molecular panel to include additional pathogens such as human metapneumovirus, adenovirus, parainfluenza viruses, and potential bacterial agents, especially in the context of syndromic surveillance and hospital case management.

The analysis of age distribution among the different viral groups revealed distinct epidemiological patterns, with important implications for surveillance strategies and clinical management. The results demonstrated a statistically significant difference in

mean ages across the respiratory virus groups, reflecting the age-specific predilection of certain viruses.

SARS-CoV-2 was predominantly detected in adults and elderly individuals, indicating a greater clinical burden in older populations. This finding aligns with existing literature, which highlights higher COVID-19 morbidity and mortality rates among elderly individuals, especially those with chronic comorbidities [1,19].

In contrast, RSV presented a mean age of 6.8 years and a median of 0.6 years, underscoring its well-established role as a leading cause of severe respiratory infections in infants and young children, particularly associated with hospitalizations for bronchiolitis and viral pneumonia [36,37].

Influenza A/B exhibited a more balanced age distribution affecting both younger adults and the elderly. This profile is consistent with the seasonal circulation of influenza viruses across broad population groups, with increased risk at the extremes of age [38,39].

HRV demonstrated the greatest age heterogeneity, with a mean age of 34.3 years and a median of 7 years, reflecting its wide distribution across children, young adults, and elderly individuals. This pattern aligns with the known year-round community circulation of HRV and its association with both mild upper respiratory infections and severe disease in high-risk individuals [40,41].

The findings emphasize the importance of age-stratified surveillance in respiratory viral epidemiology and highlight the need for prevention strategies targeted to specific age groups, including vaccination campaigns, protective measures tailored by age, and adequate resource allocation during critical seasonal periods.

The greater proportion of female cases among SARS-CoV-2 infections, as observed in this study, may reflect occupational exposure, particularly among healthcare and caregiving professionals, as suggested by Costanza et al. (2022) [42].

The predominance of cases in urban areas may be related to higher population density and exposure to high-risk transmission locations such as schools and public transportation. Several studies have demonstrated that population density is crucial in the spread of COVID-19 and other respiratory viroses [43-45].

The PCA performed in this study revealed distinct syndromic profiles associated with each viral etiology, reflecting both clinical severity and symptomatologic patterns among the studied respiratory infections.

PC1 was associated with respiratory severity markers (dyspnea, respiratory discomfort, hypoxia), with RSV showing the strongest positive loading, consistent with its role in bronchiolitis and pneumonia in children [46]. PC2 represented systemic symptoms such as fever, fatigue, and loss of smell, predominantly linked to Influenza A/B, in line with its classical febrile presentation [47]. PC3 captured a milder and heterogeneous profile, with gastrointestinal and upper respiratory symptoms; HRV loaded positively here and negatively on PC1, reflecting its generally less severe but variable clinical course [40].

SARS-CoV-2 and the group classified as SARI of undefined etiology, showed central and dispersed positions across all three components. Both groups presented negative or near-zero scores across the principal components, indicating clinical heterogeneity without a dominant symptom cluster. This dispersion may reflect the broad clinical spectrum of SARS-CoV-2, ranging from asymptomatic to severe presentations, as well as the etiological diversity within the undefined SARI group, which likely includes untested viruses and bacterial infections [48,49].

These findings demonstrate the utility of PCA as an exploratory tool for identifying syndromic signatures among respiratory viruses. They also suggest that, while classical respiratory pathogens like RSV and Influenza A/B retain well-defined clinical profiles, SARS-CoV-2 and undefined etiologies present considerable overlap and variability, posing challenges for clinical diagnosis based solely on symptomatology.

RSV positive cases showed high hospitalization and ICU rates, likely influenced by selection bias from SARI-focused sampling; population-based studies report lower rates (2–6% in infants) [24,50,51]. Nonetheless, the high demand for ventilatory support underscores RSV's clinical impact. Influenza A/B hospitalizations were also elevated, mainly in elderly patients with comorbidities, with 21.42% requiring ICU care, consistent with its known disproportionate burden in older populations [52,53]. For SARS-CoV-2, hospitalization reached 19.04%, with half requiring ICU and all needing ventilatory support, reinforcing its severity in older adults and those with chronic conditions [54-57]. HRV, despite being classically mild, had a hospitalization rate of

26.66%, with 37.5% ICU admissions, all in elderly with comorbidities, highlighting its underestimated role in severe infections [40,58-61]. The “Other SARI” group showed 42.25% hospitalization, 27.11% ICU admission, and nearly half requiring mechanical ventilation, with a mean age of 33.7 years, suggesting a heterogeneous etiology including bacterial and untested viral agents.

The results of this study reinforce the need for improvement in epidemiological surveillance and differential diagnosis of SARI in Brazil. The high proportion of cases with no specific etiological diagnosis emphasizes the importance of expanding molecular testing to include other respiratory viruses and potentially bacterial coinfections. Furthermore, the predominance of RSV as a cause of hospitalizations suggests that preventive strategies, such as vaccination against RSV in high-risk groups, should be considered in the country, following models adopted in other regions [62,63].

The irregular behavior of SARS-CoV-2 in the post-pandemic period suggests a shift in the epidemiological profile of this virus, which may be progressing toward becoming endemic, without a clear seasonality. However, the possibility of new variants emerging and altering this pattern necessitates continuous monitoring of viral circulation and its interaction with other respiratory pathogens [64].

In conclusion, this study contributes to the understanding of the dynamics of viral respiratory infections in a post-pandemic scenario and provides insights for improving diagnostic strategies, surveillance, and clinical management of these conditions.

6. Conflict of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

7. Author contributions

Conceptualization: CGD, KO, ASAW; Methodology: KO, ASAW, MCOG, LH, CGD; Formal analysis: KO, ASAW, CGD; Investigation: KO, CGD, ASAW, MCOG, LH, ABFM, VCD, VLS; Data curation: KO, ASAW, CGD, MCOG, LH; Visualization: KO, ASAW, CGD; Writing – original draft: KO, ASAW; Writing – review & editing: CGD, KO, ASAW; Supervision: CGD; Project administration: CGD, ASAW. KO; Funding acquisition: CGD.

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9. Ethics declaration

This study was approved by the Research Ethics Committee on Human Beings under protocol number 710133723.3.0000.5147. Anonymity was not assured because limited personal identifiers may be required during data collection and verification procedures. Nevertheless, confidentiality was strictly maintained. All records were de-identified and assigned a unique study identification code before analysis. The linkage key (code-to-identity file) was stored separately from the analytical dataset and was accessible only to the principal investigator and designated authorized personnel. Electronic data were stored on password-protected, access-restricted institutional computers/servers, with regular backups. Files containing sensitive information were additionally protected through restricted folder permissions and, when available,

encryption. No identifiable information is disclosed in any publication resulting from this study.

10. Acknowledgments

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Generative AI tools were not used for literature review, study design, data collection, data analysis, interpretation of results, manuscript drafting, editing, figure creation, or reference management. This research has not been previously presented, published, or disseminated in any form or through any platform.

11. Article Highlights

- Severe acute respiratory infection (SARI) surveillance was evaluated in the post-pandemic period across two Regional Health Management Offices (GRS) in Minas Gerais, Brazil (Barbacena and Leopoldina), covering 48 municipalities.
- The etiological profile of SARI was assessed focusing on routinely tested viroses, SARS-CoV-2, influenza viruses, respiratory syncytial virus (RSV), and human rhinovirus (HRV).
- A substantial proportion of SARI cases remained negative for the main tested pathogens, suggesting an etiological gap and the potential contribution of other respiratory viruses and/or pathogens.

- Clinical and epidemiological patterns were described to support more targeted diagnostic strategies and improved case management in regional health services.
- The findings reinforce the need to expand respiratory pathogen testing and strengthen integrated surveillance to better characterize SARI burden in the post-pandemic setting.

12. References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Hanage WP, Schaffner W. Burden of Acute Respiratory Infections Caused by Influenza Virus, Respiratory Syncytial Virus, and SARS-CoV-2 with Consideration of Older Adults: A Narrative Review. Infect Dis Ther. 2025;14 (Suppl 1): 5–37. Doi:10.1007/s40121-024-01080-4.

2. Pletz MW, Dürrwald R, Reiche J, et al. Impact of the COVID-19 pandemic on influenza and respiratory syncytial virus antibody titres in the community: a prospective cohort study in Neustadt, Thuringia, Germany. Eur Respir J. 2022;60(5):2200947. doi:10.1183/13993003.00947-2022.

3. Boon H, Meinders A, van Hannen EJ, et al. Comparative analysis of mortality in patients admitted with an infection with influenza A/B virus, respiratory syncytial virus, rhinovirus, metapneumovirus or SARS-CoV-2. Influenza Other Respir Viruses. 2024;18(1):e13237. doi:10.1111/irv.13237.

4. Branche A, Ramesh M, Francis B. A narrative review of key risk factors for severe illness following SARS-CoV-2, influenza virus, and respiratory syncytial virus infection. Infect Dis Ther. 2025;14(Suppl 1):39-61. doi:10.1007/s40121-024-01081-3.

5. Marmo FAD, Ikegami EM, Oliveira NGN, et al. Overview of hospitalizations for COVID-19 among aged individuals residing in Minas Gerais, Brazil. Rev Bras Geriatr Gerontol. 2023;26:e230154. doi:10.1590/1981-22562023026.230154.en.

6. Bissonnette L, Bergeron MG. Infectious disease management through point-of-care personalized medicine molecular diagnostic technologies. J Pers Med. 2012;2(2):50-70. doi:10.3390/jpm2020050.

7. Geismar C, Nguyen V, Fragaszy E, et al. Symptom profiles of community cases infected by influenza, RSV, rhinovirus, seasonal coronavirus, and SARS-CoV-2 variants of concern. Sci Rep. 2023 Aug 2;13(1):12511. doi:10.1038/s41598-023-38869-1.

8. Dutta D, Naiyer S, Mansuri S, et al. COVID-19 Diagnosis: A Comprehensive Review of the RT-qPCR Method for Detection of SARS-CoV-2. Diagnostics (Basel). 2022 Jun 20;12(6):1503. doi: 10.3390/diagnostics12061503.

9. Quintero-Salgado E, Briseno-Ramírez J, Vega-Cornejo G, et al. Seasonal Shifts in Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses After the COVID-19 Pandemic: An Eight-Year Retrospective Study in Jalisco, Mexico. Viruses. 2024Dec 8;16(12):1892. doi:10.3390/v16121892.

10. Tang JW, Lam TT, Zaraket H, et al. Global epidemiology of non-influenza RNA respiratory viruses: data gaps and a growing need for surveillance [published

correction appears in Lancet Infect Dis. 2017;17(10):e320-e326. doi:10.1016/S1473-3099(17)30238-4.

11. Gilca R, Amini R, Douville-Fradet M, et al. Other respiratory viruses are important contributors to adult respiratory hospitalizations and mortality even during peak weeks of the influenza season. Open Forum Infect Dis. 2014;1(2):ofu086. doi:10.1093/ofid/ofu086.

12. Martins RB, Rocha LP, Prates MM, et al. Respiratory DNA viruses are undetectable in nasopharyngeal secretions from adenotonsillectomized children. PloS one. 2017;12(3), e0174188. doi:10.1371/journal.pone.0174188.

13. Paul R, Arif AA, Adeyemi O, et al. Progression of COVID-19 from urban to rural areas in the United States: A spatiotemporal analysis of prevalence rates. J Rural Health. 2020 Sep;36(4):591–601. doi:10.1111/jrh.12486.

14. Kang JA, Quigley DD, Chastain AM, et al. Urban and Rural Disparities in COVID-19 Outcomes in the United States: A Systematic Review. *Med Care Res Rev*. 2025;82(2):119-136. doi:10.1177/10775587241298566

15. Instituto Todos pela Saúde (ITpS). Monitoramento de Patógenos Respiratórios – Relatório 30 [Internet]. São Paulo: ITpS; 2024 [cited 2025 nov 4]. Available from: https://www.itps.org.br/pesquisa-detalhe/monitoramento-de-patogenos-respiratorios-relatorio-30

16. Instituto Todos pela Saúde (ITpS). Monitoramento de Patógenos Respiratórios – Relatório 37 [Internet]. São Paulo: ITpS; 2024 Nov 29 [cited 2025 nov 4]. Available from: https://www.itps.org.br/pesquisa-detalhe/monitoramento-de-patogenos-respiratorios-relatorio-37

17. Kim D, Quinn J, Pinsky B, et al. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. JAMA. 2020 May 26;323(20):2085-2086. doi:10.1001/jama.2020.6266

18. Esper FP, Spahlinger T, Zhou L. Rate and influence of respiratory virus coinfection on pandemic influenza A virus infection in children. J Infect Dis. 2011 Oct;63(4):260–266. doi:10.1016/j.jinf.2011.04.004

19. Mostafa HH, Fall A, Norton JM, et al. Respiratory virus disease and outcomes at a large academic medical center in the United States: a retrospective observational study of the early 2023/2024 respiratory viral season. Microbiol Spectr. 2024 Oct;12(10):e01116-24. doi:10.1128/spectrum.01116-24

20. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045

21. Viloria Winnett A, Akana R, Shelby N, et al. Daily SARS-CoV-2 nasal antigen tests miss infected and presumably infectious people due to viral load differences among specimen types. Microbiol Spectr. 2023 Aug;11(4):e01295-23. doi:10.1128/spectrum.01295-23

22. Guo L, Deng S, Sun S, Wang X, Li Y. Respiratory syncytial virus seasonality, transmission zones, and implications for seasonal prevention strategy in China: a systematic analysis. Lancet Glob Health. 2024 Jun;12(6):e1005–e1016. doi:10.1016/S2214-109X(24)00090-1

23. Shan S, Zhang W, Gao H, et al. Global seasonal activities of respiratory syncytial virus before the coronavirus disease 2019 pandemic: a systematic review. Open Forum Infect Dis. 2024 Apr 25;11(5):ofae238. doi:10.1093/ofid/ofae238

24. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet. 2017;390(10098):946–958. doi:10.1016/S0140-6736(17)30938-8

25. Liang Y, Sun Z, Hua W, et al. Spatiotemporal effects of meteorological conditions on global influenza peaks. Environ Res. 2023;231(Pt 2):116171. doi:10.1016/j.envres.2023.116171

26. Neumann G, Kawaoka Y. Seasonality of influenza and other respiratory viruses. EMBO Mol Med. 2022 Apr;14(4):e15352. doi:10.15252/emmm.202115352

27. World Health Organization. COVID-19 - Global situation [Internet]. Geneva: World Health Organization; 2025 May 28 [cited 2025 Jun 5]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2025-DON572

28. Townsend JP, Hassler HB, Lamb AD, et al. Seasonality of endemic COVID-19. mBio. 2023 Dec 19;14(6):e0142623. doi:10.1128/mbio.01426-23

29. Centers for Disease Control and Prevention. Background for CDC's updated respiratory virus guidance [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2024 Mar 1 [cited 2025 Jun 4]. Available from: https://archive.cdc.gov/www_cdc_gov/respiratory-viruses/background/index.html

30. Tan ST, Rodríguez-Barraquer I, Kwan AT, et al. Strength and durability of indirect protection against SARS-CoV-2 infection through vaccine and infection-acquired immunity. Nat Commun. 2025 Jan 29;16(1):1090. doi:10.1038/s41467-025-42363-6

31. Thornhill EM, Verhoeven D. SARS-CoV-2 mediated inhibition of respiratory syncytial virus. bioRxiv [Preprint]. 2024 Sep 3 [cited 2025 Jun 01]. doi:10.1101/2024.09.02.610835. Available from: https://www.biorxiv.org/content/10.1101/2024.09.02.610835v1.full.pdf

32**. Baker RE, Park SW, Yang W, et al. The impact of COVID-19 nonpharmaceutical interventions on the future dynamics of endemic infections. Proc Natl Acad Sci U S A. 2020;117(48):30547-30553. doi:10.1073/pnas.2013182117

Key contribution: Provides a mechanistic, modeling-based explanation of how COVID-19 nonpharmaceutical interventions can reshape the subsequent dynamics of endemic respiratory infections, supporting post-pandemic shifts in circulation patterns.

33. Rodrigues AF, Santos AM, Ferreira AM, et al. Year-Long Rhinovirus Infection is Influenced by Atmospheric Conditions, Outdoor Air Virus Presence, and Immune System-Related Genetic Polymorphisms. Food Environ Virol. 2019;11(4):340-349. doi:10.1007/s12560-019-09397-x

34. Mackay IM. Human Rhinoviruses: The Cold Wars Resume. J Clin Virol. 2008; 42 (4): 297–320. doi:10.1016/j.jcv.2008.04.002

35*. De Assis Carvalho EM, Ozanic K, Machado AFB, et al. Respiratory Virus Circulation during Pandemic: Why Were Some Viruses Still Circulating? Braz J Microbiol. 2025;56(3):1483-1494. doi:10.1007/s42770-025-01411-0

Key contribution: Discusses why selected respiratory viruses continued circulating during the pandemic period, offering contextual evidence to interpret heterogeneous post-pandemic reemergence patterns.

36. Suh M, Movva N, Jiang X, et al. Respiratory Syncytial Virus Is the Leading Cause of United States Infant Hospitalizations, 2009-2019: A Study of the National (Nationwide) Inpatient Sample. J Infect Dis. 2022;226(Suppl 2):S154-S163. doi:10.1093/infdis/jiac120

37. World Health Organization. Respiratory syncytial virus (RSV) [Internet]. Geneva: World Health Organization; 2025 Dec 19 [cited 2025 Jul 16]. Available from: https://www.who.int/news-room/fact-sheets/detail/respiratory-syncytial-virus-(rsv)

38. Caini S, Kroneman M, Wiegers T, et al. Clinical characteristics and severity of influenza infections by virus type, subtype, and lineage: a systematic literature review. Influenza Other Respir Viruses. 2018;12(6):780-792. doi:10.1111/irv.12575

39. Paget J, Spreeuwenberg P, Charu V, et al. Global mortality associated with seasonal influenza epidemics: new burden estimates and predictors from the GLaMOR Project. J Glob Health. 2019;9(2):020421. doi:10.7189/jogh.09.020421

40*. Jacobs SE, Lamson DM, St George K, et al. Human rhinoviruses. Clin Microbiol Rev. 2013;26(1):135–62. doi.org/10.1128/CMR.00077-12

Key contribution: Provides a comprehensive clinical and virological overview of human rhinoviruses, supporting background interpretation of HRV detection and disease relevance.

41. Monto AS. Epidemiology of viral respiratory infections. Am J Med. 2002;112 (Suppl 6A):4S–12S.

42. Costanza G, Paba P, Ciotti M, et al. Infection rate of respiratory viruses in the pandemic SARS-CoV-2 period considering symptomatic patients: two years of ongoing observations. Biomolecules. 2022;12(7):987. doi:10.3390/biom12070987

43. Silwangani B. An analysis of factors behind variations in COVID-19 infections between urban and rural areas of Zimbabwe in 2020. Texila Int J Acad Res. 2024;11(3):Art008. doi:10.21522/TIJAR.2014.11.03.Art008

44. Vargas Molina SA, Barrionuevo JFS, Perles Roselló MJ. Effect of urban structure, population density and proximity to contagion on COVID-19 infections during the SARS-CoV-2 Alpha and Omicron waves in Málaga, Spain, March 2020 to December 2021. Euro Surveill. 2025;30(3):10.2807/1560-7917.ES.2025.30.3.2400174. doi:10.2807/1560-7917.ES.2025.30.3.2400174

45. Kadi N, Khelfaoui M. Population density, a factor in the spread of COVID-19 in Algeria: statistic study. Bull Natl Res Cent. 2020;44(1):138. doi:10.1186/s42269-020-00393-x

46. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med. 2009;360(6):588-598. doi:10.1056/NEJMoa0804877

47. Eccles R. Understanding the symptoms of the common cold and influenza. Lancet. Infect Dis. 2005;5(11), 718–725.

48. Rello J, Storti E, Belliato M, et al. Clinical phenotypes of SARS-CoV-2: implications for clinicians and researchers. Eur Respir J. 2020;55(5):2001028. doi:10.1183/13993003.01028-2020

49. Zoting A, Bhise S, Mategadikar P, et al. Etiological Profile of Hospitalized Severe Acute Respiratory Infection (SARI) Patients During the COVID-19 Pandemic: A Cross-Sectional Study. Cureus. 2025;17(3):e80889. doi:10.7759/cureus.80889

50. van Summeren JJGT, Rizzo C, Hooiveld M, et al. Evaluation of a standardised protocol to measure the disease burden of respiratory syncytial virus infection in young children in primary care. BMC Infect Dis. 2021;21(1):705. doi:10.1186/s12879-021-06397-w

51. Kim T, Choi SH. Epidemiology and disease burden of respiratory syncytial virus infection in adults. Infect Chemother. 2024;56(1):1-12. doi:10.3947/ic.2024.0011

52. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA. 2003;289(2):179–86. oi:10.1001/jama.289.2.179

53. Centers for Disease Control and Prevention (CDC). Estimates of deaths associated with seasonal influenza — United States, 1976–2007. MMWR Morb Mortal Wkly Rep. 2010;59(33):1057–62.

54. Centers for Disease Control and Prevention (CDC). Underlying Medical Conditions Associated with Higher Risk for Severe COVID-19: Information for Healthcare Professionals. 2023. Available from: https://www.cdc.gov/covid/hcp/clinical-care/underlying-conditions.html

55. Wang B, Li R, Lu Z, et al. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. Aging (Albany NY). 2020;12(7):6049-6057. doi:10.18632/aging.103000

56. Shnayder M, Slutsky AS, Brochard L. COVID-19 in patients with chronic respiratory diseases. Nature Medicine. 2022;28:575–576.

57. Allegra A, Yu G, El-Helou G, et al. Clinical characteristics and outcomes of patients in ICUs in New York and Lombardy during the COVID-19 pandemic: A comparative study. BMC Medicine. 2021;19:231.

58**. Hung IFN, Zhang AJ, To KKW, et al. Unexpectedly high morbidity and mortality in hospitalized elderly patients associated with rhinovirus compared with influenza virus respiratory tract infection. Int J Mol Sci. 2017;18(2):259. doi:10.3390/ijms18020259

Key contribution: Demonstrates that rhinovirus infection is associated with substantial morbidity and mortality among hospitalized adults, reinforcing HRV as a clinically relevant pathogen beyond “common cold” paradigms.

59. Morelli T, Freeman A, Staples KJ, Wilkinson TMA. Hidden in plain sight: the impact of human rhinovirus infection in adults. Respir Res. 2025;26(1):120. doi:10.1186/s12931-025-03178-w

60. Bahabri I, Abdulaal A, Alanazi T, et al. Characteristics, Management, and Outcomes of Community-Acquired Pneumonia Due to Human Rhinovirus-A Retrospective Study. Can Respir J. 2022;2022:1349994. Published 2022 Dec 9. doi:10.1155/2022/1349994

61. Souza AP, Lima M, Rocha RS, et al. In-hospital outcomes of rhinovirus and respiratory syncytial virus infections in adults: a comparative study from Brazil. Infect Dis Clin Pract. 2025;33(2):e13-e18.

62*. Soares TN, Andrade NCO, Santos SDSD, et al. Risk of Incidence and Lethality by Etiology of Severe Acute Respiratory Syndrome in Hospitalized Children Under 1 Year of Age in Brazil in 2024: A Cross-Sectional Study. Trop Med Infect Dis. 2025;10(6):168. doi:10.3390/tropicalmed10060168

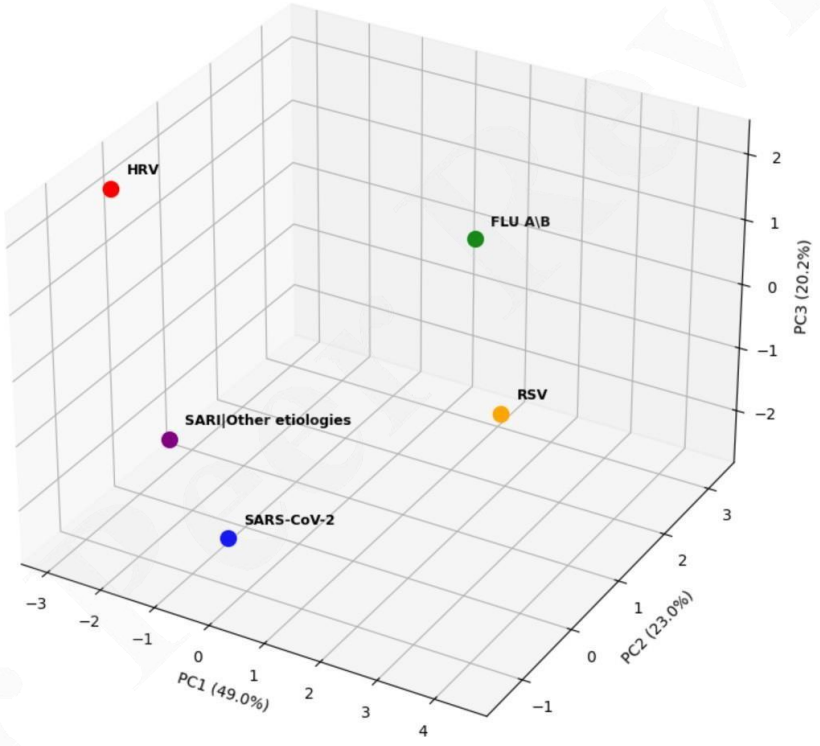
Key contribution: Supplies recent Brazilian data on etiology-specific incidence and lethality in hospitalized pediatric severe acute respiratory syndrome, supporting national contextualization of high-risk groups.

63*. World Health Organization. (2025). Progress of respiratory syncytial virus surveillance and disease burden estimation based on the Global Influenza Surveillance and Response System: Meeting report, Lima, Peru, 22–26 April 2024 **[Internet]**. Geneva: World Health Organization; 2025 Jan 13 **[cited 2025 Jun 20]**. Available from: WHO IRIS.

Key contribution: Summarizes WHO progress and standardization efforts for RSV surveillance and disease-burden estimation within GISRS, strengthening the surveillance and policy implications of the study.

64 Manirambona E, Okesanya OJ, Olaleke NO, et al. Evolution and implications of SARS-CoV-2 variants in the post-pandemic era. **Discov Public Health.** 2024;21:16. doi:10.1186/s12982-024-00140-x

List of Figures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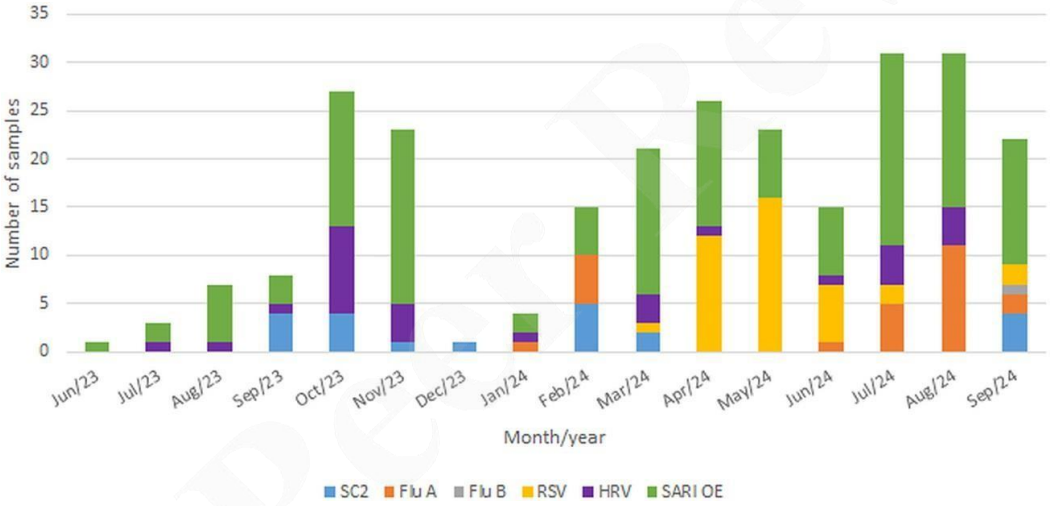

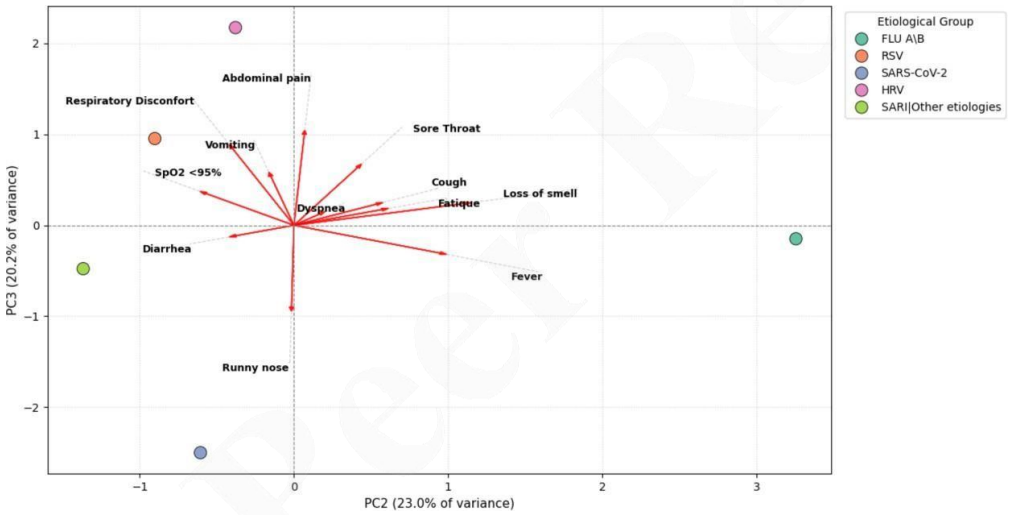

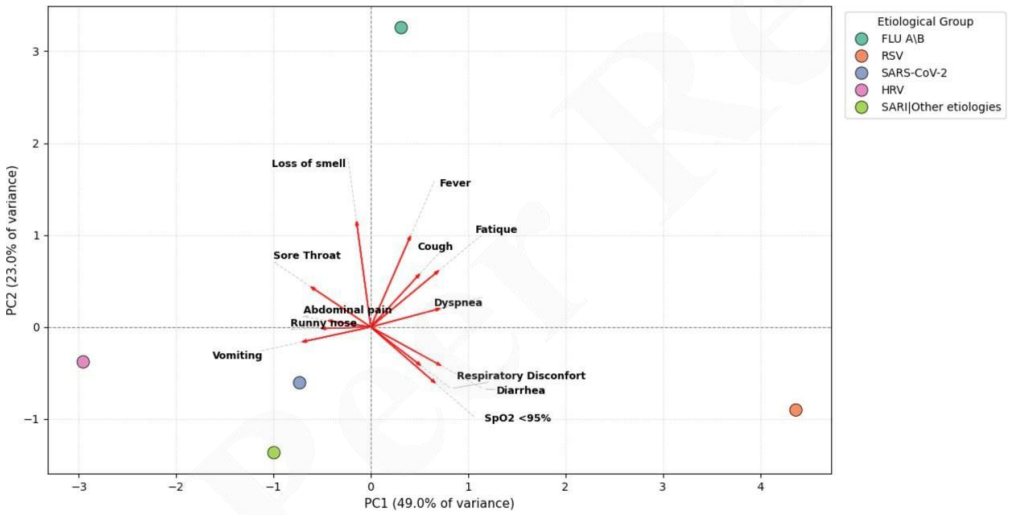

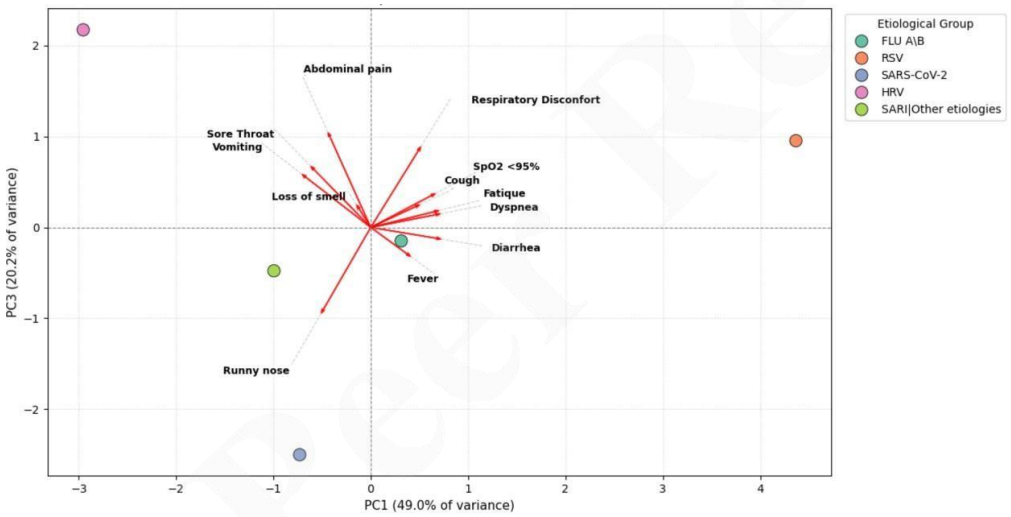

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CAPÍTULO 3:

LONG COVID: GENERAL PERCEPTIONS AND CHALLENGES IN DIAGNOSIS AND MANAGEMENT

COVID

MDPI

Review

Long COVID: General Perceptions and Challenges in Diagnosis and Management

Katia Ozanic, Aripuana Sakurada Aranha Watanabe, Alesandra Barbosa Ferreira Machado, Vania Lucia da Silva, Vanessa Cordeiro Dias and Claudio Galuppo Diniz *

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Abstract: On 11 March 2020, the World Health Organization (WHO) declared a pandemic caused by SARS-CoV-2, raising global health concerns. Reports of persistent and new symptoms following the acute phase of infection highlighted the complexities of recovery and prompted the investigation of what is now termed long COVID. Officially recognized by the WHO in October 2021, long COVID presents various health implications, though the terminology—such as post-COVID syndrome and post-acute sequelae of COVID-19 (PASC)—remains inconsistent, complicating diagnostic standardization. Long COVID affects an estimated 10% to 30% of SARS-CoV-2-infected individuals, with common symptoms including fatigue, dyspnea, cognitive dysfunction, and joint pain, all of which significantly impair quality of life. Public perception is influenced by factors like education and health history, while misinformation and stigma hinder accurate diagnosis and treatment. The absence of biomarkers and overlap with other post-viral syndromes further complicate clinical recognition. Experts emphasize the need for refined diagnostic criteria and integrated strategies combining biomedical research, public policy, and educational initiatives to improve clinical management, address healthcare inequalities, and mitigate the impacts of long COVID. This review unveils the state of the art and knowledge gaps to encourage discussion, with the aim of achieving better clinical decision-making and public awareness related to long COVID.

Keywords: long COVID; post-COVID syndrome; public perception; persistent symptoms; knowledge gaps; health policy

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1. Introduction

Long COVID, also referred to as post-COVID syndrome or long COVID syndrome, is a complex condition characterized by persistent symptoms that continue or emerge after the acute phase of SARS-CoV-2 infection, even in mild and asymptomatic cases [1]. These symptoms can persist for weeks or months and affect various body systems, complicating the recovery process for patients [2,3]. The most common symptoms include fatigue, dyspnea, cognitive dysfunction, chest pain, and joint pain [4,5].

Research by Sudre [6] indicates that long COVID affects, on average, between 10% and 30% of individuals who have had COVID-19. Estimates of long COVID prevalence range from 2.0% to 53% (7.5% to 53% in adults and 2% to 3.5% in pediatric cases) [7]. These variations reflect not only diverse methodologies but also a lack of consensus on the operational definition of the condition. Methodological differences include variations in diagnostic criteria, populations studied, and symptom measurement approaches. This

wide range underscores the need for the standardization of definitions and study methods to achieve more accurate and globally comparable estimates. Various studies suggest that prevalence is higher among women, older individuals, and those with pre-existing conditions [8–10]; however, in countries with limited healthcare access, the prevalence may be underestimated, complicating a uniform global assessment [11]. Furthermore, observed discrepancies highlight the urgency for additional research to clarify the true magnitude of long COVID across different epidemiological and socioeconomic contexts.

Long COVID extends beyond physiological effects, significantly impacting the societal, emotional, and financial dimensions of individuals, who often struggle to recognize and attribute their symptoms to the condition. Many individuals encounter obstacles in obtaining diagnoses and assistance within healthcare systems [7,12]. Marginalized populations, including informal workers, face substantial challenges in diagnosing and managing long COVID due to their limited access to formal healthcare systems. Financial and institutional barriers hinder their ability to seek medical care, while job insecurity and the lack of employment support exacerbate recovery difficulties, reinforcing the socioeconomic impacts of the condition. These barriers can lead to underreporting of cases and significant delays in diagnosis, perpetuating inequality in access to treatment and long-term medical follow-up [7].

Public perception regarding long COVID plays a crucial role in seeking medical intervention and adhering to therapeutic regimens. Misinformation and stigma associated with the condition often hinder diagnosis and the initiation of treatment, exacerbating physical, emotional, and social ramifications [13]. To address these challenges, investment in educational initiatives and integrated public health strategies is imperative to raise awareness and adapt healthcare systems to meet the unique needs of affected individuals [2]. These strategies can improve the quality of life of these individuals and reduce pressure on medical services [2,13]. In addition, there is a lack of understanding regarding the mechanisms underlying long COVID, and its similarities to other post-viral syndromes may contribute to public confusion and misconceptions [2,14]. Additionally, the overlap of symptoms with other prolonged viral infections, such as sequelae from Epstein–Barr and influenza infections, complicates diagnosis and creates uncertainties surrounding the optimal management of long COVID [15].

The aim of this review is to examine factors that shape societal perceptions of long COVID, particularly given the rising prevalence of cases characterized by persistent and incapacitating symptoms. Our objectives also include the evaluation of remaining knowledge gaps related to long COVID, such as the absence of diagnostic biomarkers and symptom heterogeneity, which hinder clinical identification of the condition and directly affect patients' perceptions and trust in their diagnoses. Understanding these factors is crucial to inform future research, public policies, and educational initiatives, ultimately enhancing public awareness and improving clinical management and patient support.

2. Material and Methods

Publication period: Studies published between January 2020 and December 2024 were included, considering the relevance of the pandemic period for understanding long COVID and the increase in publications related to the topic during this period. Despite its well-defined criteria and a more rigorous approach, the present study is classified as a narrative review.

Databases: The search was carried out in the PubMed, Web of Science, and SciELO databases, chosen for their relevance to the medical literature, the interdisciplinary scope, public health, biomedicine, and social sciences and because they allow access access to publications from different regions and languages. The selection of these databases was

based on their ability to index high-quality studies, covering regional and international publications that allow for contextual and comparative analysis of the data. In addition, these databases include a diversity of languages and address different methodological approaches, ensuring the inclusion of literature that reflects the multiple dimensions of long COVID. Relevant studies found outside these platforms were considered upon explicit inclusion in the search criteria.

Language: Articles published in English and Portuguese were selected, with the aim of allowing an accessible and regionally representative analysis, while recognizing the limitation of excluding other languages.

Thematic focus: Studies addressing the prevalence, symptoms, underlying mechanisms, social impacts, and public perception of long COVID were prioritized, considering their relevance to the research questions and identified knowledge gaps.

Robust methodology: Only studies with clear designs and consistent methodologies were included. Anecdotal reports and studies with low methodological quality were excluded to ensure the validity of the conclusions.

Scientific relevance: Studies that had a significant impact on the current literature and that contributed to expanding our understanding of long COVID were selected, prioritizing those that addressed critical gaps in scientific knowledge.

Descriptors and search terms: long COVID, post COVID condition, public perception, prolonged viral infections, persistent symptoms, and knowledge gaps.

Analysis and interpretation: A critical analysis of the information collected was carried out, identifying patterns, trends, gaps in knowledge, and conflicts between studies. The data were interpreted according to their specific perspective and context.

3. Long COVID Overview

Long COVID, also known as post-acute sequelae of SARS-CoV-2, is a complex and multifaceted condition that affects a subset of individuals infected with the SARS-CoV-2 virus, the causative agent of COVID-19. This condition is characterized by the persistence of symptoms for an extended duration, often lasting several months or even years after the initial infection has resolved [16,17].

The primary symptoms associated with long COVID include fatigue, shortness of breath, cognitive dysfunction, and a wide range of other physical and neurological manifestations [18,19]. These symptoms can significantly impact the quality of life of affected individuals, with some experiencing substantial declines in overall health and well-being [16].

To diagnose long COVID, healthcare providers typically rely on a comprehensive clinical evaluation that includes a thorough medical history, physical examination, and various diagnostic tests [19]. These tests may encompass imaging studies, such as chest X-rays or CT scans, to assess lung damage, as well as neurological and cognitive assessments to evaluate the impact on the nervous system. Additionally, laboratory tests may be conducted to rule out other underlying conditions or to identify potential biomarkers associated with long COVID [16,17,20].

3.1. Prevalence and Symptom Variability

Recent studies have highlighted the prevalence of long COVID, with community-based surveys indicating that 15–18% of patients continue to experience symptoms beyond the initial 4-week period, and 8–17% beyond 12 weeks [21,22]. In a population-based study, 15.9% of participants reported new or worsened symptoms at least 90 days after infection, with higher prevalence linked to specific acute symptoms [22]. Moreover, a global cohort study found that the prevalence of long COVID was notably higher in high-income countries compared to low- and middle-income countries, suggesting disparities in health

outcomes and support systems [11]. The risk of developing long COVID is particularly high among those who experienced more severe illness, with estimates indicating a more than twofold increase in risk for individuals who required hospitalization or admission to intensive care units [19]. Servier (2023) [23] describes that 91% of the patients studied showed a gradual and slow improvement within 2 years of the syndrome course, and within this population, 5% improved rapidly and 4% presented a persistent condition. Based on these data, spontaneous resolution in long COVID does not appear to be a frequent phenomenon.

The symptoms associated with long COVID are highly diverse, encompassing a range of physiological and neurological manifestations. Respiratory complications have been widely documented, underscoring the lasting impact of COVID-19 on respiratory health [19,24]. Extrapulmonary effects and neuromuscular dysfunction further highlight the systemic nature of the condition [19]. The main symptoms and related organs and systems affected are presented in Figure 1.

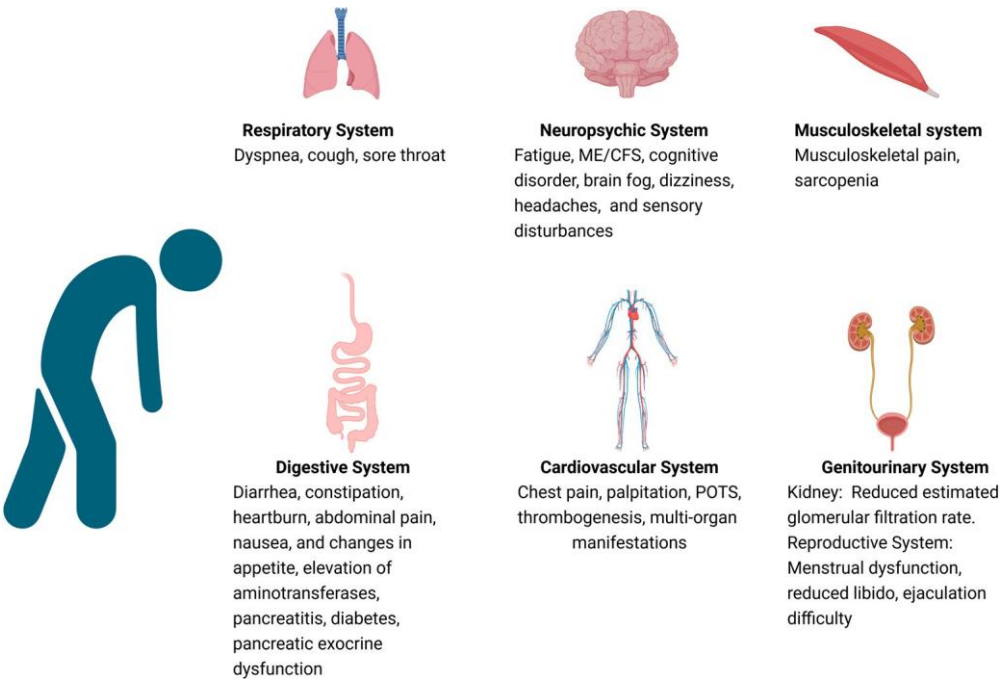

Figure 1. Long COVID is a multi-system condition that can impact different organs, including the respiratory, cardiovascular, neuropsychic, digestive, circulatory, musculoskeletal, and genitourinary systems. It is also associated with conditions such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and postural orthostatic tachycardia syndrome (POTS). Adapted from Li (2023) [25]. Created with BioRender: https://www.biorender.com/ (accessed on 3 March 2025).

Differences in the reported variability are attributed to a lack of consensus on diagnostic criteria, as well as methodological variations and differences in the populations studied [26,27]. Additionally, regional disparities, such as lower recognition and underreporting in countries with limited healthcare access, exacerbate this complexity [28].

3.2. Public Perception of Long COVID

The perception of long COVID can be influenced by various factors, including education level, age, health history, and access to information about the condition [18,29]. Individuals with higher levels of education may be better equipped to understand the complex and evolving nature of long COVID, as research indicates that more educated populations tend to have a more nuanced comprehension of health conditions [26]. This

may lead to a more informed and less anxious perception of long COVID compared to those with limited educational backgrounds, who may struggle with the intricacies of the condition.

Age is another significant factor that shapes perceptions of long COVID. Older adults, who are generally more vulnerable to severe outcomes from COVID-19, may view long COVID as a more substantial threat, while younger individuals might underestimate the potential long-term implications of the disease. Furthermore, individuals with pre-existing health conditions, such as respiratory or cardiovascular diseases, may be more attuned to the potential long-term effects of COVID-19, resulting in heightened awareness and concern regarding long COVID [19,20].

3.3. Impact of Media and Social Networks

The COVID-19 pandemic has profoundly impacted public health and society [27]. The role of media and social networks in shaping the public understanding and perception of long COVID is crucial, as both accurate information and misinformation can significantly influence people's knowledge and attitudes toward this syndrome [27].

Research has shown that social media usage has increased dramatically during the pandemic, leading to the dissemination of both reliable information and misinformation about COVID-19 and its long-term effects [28]. Social media platforms have become primary sources of health information for many individuals, and the abundance of content—some of which may be inaccurate or misleading—has contributed to what has been termed an "infodemic" [29,30].

Studies indicate that social media is often utilized as a primary source of health information, particularly in contexts where access to formal medical resources is limited. For example, analyses of Twitter data have revealed prevalent discussions of symptoms such as "brain fog" and fatigue, contributing to the collective recognition of long COVID manifestations. However, content posted on social media may include unverified information, creating confusion and fueling misinformation [31].

For instance, users of platforms like TikTok have demonstrated less knowledge of COVID-19 guidelines, which may extend to misconceptions about long COVID symptoms [32]. Conversely, another study utilizing Twitter data analyzed discussions about long COVID, revealing prevalent symptoms such as brain fog and fatigue, thereby demonstrating how social media can facilitate our understanding of long COVID experiences [31]. Overall, the relationship between social media use and knowledge of long COVID is complex, necessitating targeted public health interventions to mitigate misinformation and improve our understanding [33].

Thus, social media represents a critical space for shaping public perceptions of long COVID, highlighting the need to balance the dissemination of accurate information with the mitigation of misinformation. Strategic engagement by healthcare institutions on these platforms can play a vital role in educating the public and enhancing health responses.

3.4. Scientific Knowledge Gaps Regarding Long COVID

The COVID-19 pandemic has left an indelible mark on the global health landscape, and the emergence of long COVID has become a significant area of concern. The clinical spectrum of long COVID is characterized by a diverse array of symptoms that often persist long after the initial infection has resolved [34,35]. However, the underlying mechanisms and long-term prognosis of this condition remain poorly understood, leading to significant gaps and controversies in current scientific research [5,36].

One of the primary challenges in understanding long COVID is the lack of consensus on the pathophysiological mechanisms driving persistent symptoms. Hypothesized

mechanisms include end-organ damage, specific symptom clusters, and potential autoimmune responses, but the precise pathways remain elusive [37]. The heterogeneity of long COVID presentations further complicates efforts to establish a coherent schema linking SARS-CoV-2 infection to the diverse array of persistent symptoms [37].

Despite the lack of definitions on the pathophysiological mechanisms of long COVID, some studies indicate possible explanations for symptoms such as chest pain and brain fog. Microvascular dysfunction appears to play a central role in persistent chest pain observed in patients with long COVID. Cardiac magnetic resonance imaging with stress perfusion revealed that 50% of the patients evaluated presented a circumferential subendocardial perfusion defect pattern, highly suggestive of microvascular ischemia. This finding suggests that chest pain in these individuals may be associated with endothelial dysfunction induced by SARS-CoV-2, leading to inflammation and impairment of the coronary microcirculation, even in the absence of epicardial coronary artery disease.

Thus, the evaluation and management of chest pain in long COVID should consider microangiopathy as a relevant etiological factor, requiring specific diagnostic and therapeutic approaches for microvascular dysfunction [38]. Microvascular dysfunction also plays a central role in the development of brain fog in patients with long COVID. Chronic vascular inflammation and blood–brain barrier (BBB) dysfunction may allow pro-inflammatory cytokines to enter the central nervous system, triggering neuroinflammation and compromising cerebral perfusion. The reduction in blood flow may affect the delivery of oxygen and nutrients to neurons, resulting in cognitive symptoms such as difficulty concentrating, memory lapses, and persistent mental fatigue.

Furthermore, microangiopathy may contribute to a state of chronic cerebral hypoperfusion, similar to that observed in neurodegenerative diseases, exacerbating cognitive deficits. Therapeutic strategies aimed at microvascular regeneration and controlling inflammation may be key to mitigating the effects of brain fog in patients with long COVID [39]. Possible pathophysiological mechanisms are presented in Figure 2.

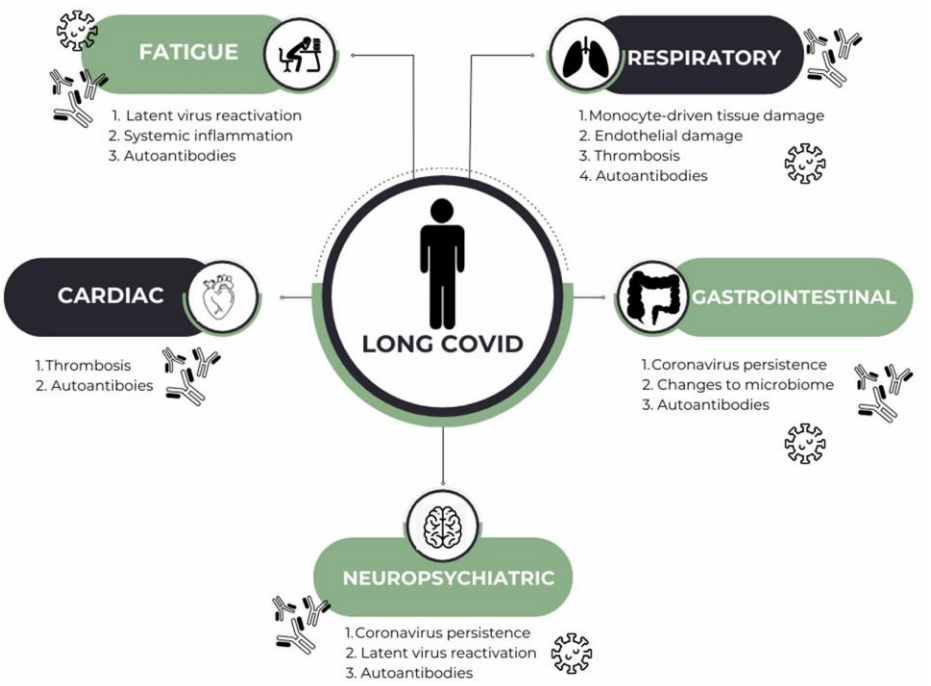

Figure 2. Common symptoms associated with long COVID and possible underlying pathophysiology. Adapted from Liew (2023) [40]. Created with Canva: www.canva.com (accessed on 3 March 2025).

Additionally, the duration of long COVID is another aspect that remains highly debated. While some individuals may experience a gradual recovery, others may suffer from recurring or worsening symptoms, leading to a prolonged and unpredictable course of the disease [5].

Limitations in current scientific research on long COVID can be attributed to several factors, including the evolving nature of the disease and the absence of a clear, universally accepted definition. These challenges have hampered the development of standardized diagnostic criteria and effective treatment approaches [26,34,35]. Furthermore, the paucity of well-characterized clinical cohorts and the need for more rigorous scientific investigations have impeded progress in understanding the underlying mechanisms and risk factors associated with long COVID [41].

Alterations in the immune systems of individuals with long COVID have been widely documented, revealing complex immune profiles and persistent inflammatory responses. Studies indicate that patients with long COVID exhibit elevated levels of various leukocytes, including lymphocytes, eosinophils, and monocytes, which correlate with the duration and severity of symptoms [42]. Additionally, distinct inflammatory groups have been identified, such as limited and systemic immune activation, which are associated with clinical variables like age and vaccination status [43]. Furthermore, immune dysregulation is evident through intensified antibody responses not only to SARS-CoV-2 but also to other viruses, suggesting a compromised viral control mechanism [44]. Single-cell RNA sequencing has shown changes in immune cell populations over time, indicating ongoing challenges in virus clearance and possible chronic complications [45]. Overall, these findings underscore the intricate immune alterations that characterize long COVID, affecting a significant portion of the global population [46].

In patients with long COVID, persistent oxidative stress and mitochondrial dysfunction significantly contribute to cellular damage and metabolic changes, alongside immune dysregulation. Evidence indicates that mitochondrial dysfunction leads to impaired cellular energy metabolism, characterized by increased glycolysis and oxidative phosphorylation, disrupting normal cellular functions and promoting apoptotic activity [47,48]. Additionally, the metabolic profiles of patients with long COVID reveal low levels of amino acids and triglycerides, suggesting mitochondrial stress and altered metabolic pathways linked to chronic fatigue syndrome [49]. The interaction between oxidative stress and metabolic disorders exacerbates symptoms such as fatigue and cognitive disturbances, highlighting the need for targeted therapeutic strategies that address these underlying metabolic issues [50]. Therefore, understanding these mechanisms is crucial for developing effective interventions for long COVID.

Another question that remains unanswered is whether long COVID is a syndrome or a combination of more localized diseases that, when combined, culminate in a systemic condition. This definition is important, especially for diagnostic and treatment aspects, as each definition results in different consequences and outcomes.

3.5. Consequences of Lack of Specific Knowledge and Confusion with Other Viral Infections

The ambiguity surrounding long COVID has significantly contributed to public misconceptions regarding its severity and impact. Two confounding factors may complicate the clinical and laboratory diagnosis of long COVID: a wide range of symptoms and a lack of understanding of the underlying mechanisms of the disease [51,52]. Furthermore, the interplay of comorbidities and immune dysregulation has been identified as critical in understanding the trajectory of long COVID, necessitating multidisciplinary approaches for effective management [53]. The absence of a standardized definition and clear prognostic factors perpetuates skepticism surrounding the condition, making it difficult to recognize

the debilitating effects of long COVID on quality of life [51]. Therefore, continued research is essential to bridge these gaps and enhance public understanding of this emerging health challenge.

Prolonged symptoms following viral infections, such as those seen in influenza and Epstein–Barr virus (EBV), exhibit notable similarities with long COVID, potentially leading to confusion among patients and healthcare providers. Both conditions are characterized by a range of persistent symptoms, including fatigue, cognitive impairment, and musculoskeletal pain, which can last for months or even years post-infection [54,55].

For instance, studies indicate that post-viral fatigue is a common feature in both long COVID and post-EBV conditions, with chronic symptoms reported in a significant percentage of affected individuals [55]. Furthermore, the pathophysiological mechanisms underlying these syndromes may overlap, involving immune dysregulation and systemic effects that complicate diagnosis and treatment [56]. The cumulative prevalence of long COVID symptoms is reported to be significantly higher than that of other post-viral syndromes; however, the symptomatology often overlaps, necessitating careful clinical evaluation to differentiate between these conditions [54,57].

Vivaldi [58] compared the long-term symptom profiles of SARS-CoV-2 and other acute respiratory infections (ARIs), highlighting the substantial and diverse symptom burden associated with both types of infections. Their findings showed that both SARS-CoV-2 and non-COVID-19 ARIs resulted in a wide range of persistent symptoms and reductions in health-related quality of life (HRQoL). However, individuals with SARS-CoV-2 infection exhibited a significantly higher prevalence of certain symptoms compared to those with non-COVID-19 ARIs, including taste and smell disturbances, dizziness, hair loss, unusual sweating, heart palpitations, and memory problems.

Approximately 22% of participants with either SARS-CoV-2 or non-COVID-19 ARIs fell into the category of severe symptoms, underscoring the significant impact of these infections. Neurocognitive symptoms, such as memory problems and difficulty concentrating, were particularly prominent in individuals with SARS-CoV-2 infection. While some symptoms, including coughing and taste or smell disturbances, diminished after 12 weeks, others persisted, with more severe acute infections associated with a higher prevalence and intensity of ongoing symptoms.

Latent class analysis (LCA) identified three symptom severity clusters for SARS-CoV-2: mild (45%), moderate (32%), and severe (22%). Individuals in the severe group were characterized by a predominance of neurocognitive symptoms and were more likely to report suspected long COVID. Similar severity-based clusters were observed for non-COVID-19 ARIs, with some individuals in the severe category misattributing their symptoms to long COVID. These findings suggest that the severity of symptoms, rather than the type of infection, may be a critical determinant of long-term impact.

The study emphasizes the need for greater recognition and understanding of long-term sequelae associated with both SARS-CoV-2 and other ARIs. Persistent symptoms following non-COVID-19 ARIs may be underdiagnosed, highlighting the importance of extending research and clinical attention beyond long COVID. While the study underscores the value of severity-based symptom clusters in informing clinical care and predicting recovery trajectories, it also calls for further investigation into the mechanisms underlying these conditions to improve diagnosis and treatment.

The study's strengths include its large, community-based cohort, which captured a broad spectrum of infection severities, and the use of a contemporaneous control group to contextualize the findings. However, limitations such as reliance on self-reported symp- toms, potential recall bias, and the lack of pathogen-specific data for non-COVID-19 ARIs should be addressed in future research. Overall, the findings highlight the extensive reach of long COVID and the overlooked burden of other respiratory infections, underscoring the need for comprehensive approaches to post-acute care.

3.6. Implications for Diagnosis and Treatment

Differentiating long COVID from other similar syndromes poses significant challenges for healthcare providers and patients, impacting the perceived legitimacy of the condition. The overlap of symptoms of long COVID with other post-viral syndromes, such as chronic fatigue syndrome (ME/CFS), makes it difficult to correctly distinguish the two conditions, which can result in inaccurate diagnosis and inappropriate treatment [59,60]. The presence of comorbidities and immune dysregulation further blurs the lines, as autoantibodies and cytokine imbalances can mimic or exacerbate symptoms of other conditions [53]. This diagnostic ambiguity can lead to skepticism regarding the legitimacy of long COVID, as patients may struggle to have their experiences validated within the medical community [60].

The lack of diagnosis and recognition of long COVID can have far-reaching consequences. Patients may encounter significant challenges in accessing appropriate healthcare and support, leading to delays in treatment and prolonged recovery processes. Furthermore, the absence of robust epidemiological data on the prevalence and burden of long COVID hinders the development of targeted interventions and resource allocation, ultimately impacting the overall public health response [11].

Moreover, the evolving nature of diagnostic criteria and the reliance on subjective evidence from patients contribute to the ongoing debate regarding long COVID's recognition as a distinct clinical entity, necessitating further research and multidisciplinary approaches for effective management [61,62]. Scientific evidence underscores the urgent need for increased awareness, improved diagnostic tools, and the development of comprehensive healthcare strategies to address the challenges posed by long COVID [53,62].

A systematic review published at the end of 2024 listed 24 clinical trials with possible treatments for long COVID. Treatments range from drug interventions to physical activity, rehabilitation, and behavioral treatments [23]. Despite the number of studies in this field, there is currently no effective and approved treatment for the syndrome. The lack of a well-defined diagnosis, associated with physicians' lack of knowledge, can hinder clinical management and also possible treatments.

Another possible line of treatment involves modifying or adapting treatments already approved for acute COVID. For example, a study published in 2025 suggests that some cases of long COVID may benefit from prolonged treatment with the nirmatrelvir/ritonavir combination [63].

A major issue in the treatment of long COVID is the understanding that the symptoms are independent and caused by different diseases, therefore doctors only treat the symptoms and often do not address the cause of the problems. Regardless of the line of treatment, there is an urgent need for more studies that can help patients in a more holistic way.

4. Concluding Remarks

The literature observations in this study underscore the inherent complexity of long COVID, both in terms of its clinical definition and its broader social and public health implications. As a multifaceted condition, long COVID encompasses a wide range of persistent symptoms affecting multiple body systems, complicating diagnosis and clinical management. The lack of specific biomarkers and standardized diagnostic criteria contributes to underreporting and hinders recognition of its severity, particularly in populations with limited access to healthcare services.

Public perception of long COVID emerges as a critical factor influencing both the pursuit of medical care and adherence to treatment. However, conflicting information, misinformation, and stigma continue to negatively affect how this condition is understood and managed. This situation underscores the need for educational campaigns designed to enhance our understanding of long COVID, reduce stigma, and promote early diagnosis and effective treatment.

Furthermore, the similarities between long COVID and other post-viral syndromes, such as those associated with Epstein–Barr virus and influenza, highlight the challenges faced by healthcare providers and patients in differentiating these conditions. This overlap may impact the perceived legitimacy of long COVID, restricting adequate resource allocation for its management. Investment in research aimed at exploring distinctive features and underlying pathophysiological mechanisms is essential to overcome these barriers.

The knowledge gaps identified emphasize the need of collaborative efforts to develop multidisciplinary approaches that integrate biomedical research, health policies, and educational interventions. Such efforts have the potential to improve the clinical management of long COVID and strengthen healthcare systems in addressing similar conditions.

Finally, the data highlight that effectively addressing the implications of long COVID requires actions that extend beyond the medical realm, incorporating social and economic dimensions. Investments in public awareness, psychological support, and workplace adaptations can mitigate the prolonged impact of the disease and promote more comprehensive recovery for affected individuals. It is imperative to continue exploring the intersections between research, clinical practice, and public policy to tackle the challenges posed by long COVID effectively.

5. Future Directions

Several aspects of long COVID still require further studies to better understand the syndrome, possibly resulting in better diagnosis and treatment. Clinical studies to assess viral persistence and drug treatment and understand the pathophysiology of the disease are urgently needed. Follow-up studies of patients over long periods of time may demonstrate the evolution of the natural history of the disease, allowing possible risk factors to be identified. An analysis of biomarkers that can safely and accurately identify long COVID has not yet been conducted, and this information is extremely important for definitive diagnosis and treatment, if applicable. Comparing long COVID with other persistent viral syndromes may answer questions such as possible pathophysiological mechanisms and viral reservoirs. Although many questions that have arisen in recent months are yet to be answered, there have been considerable advances in knowledge about the syndrome and its pathogenic mechanisms and possible treatments. Long COVID is a mass disabling event and urgently needs the attention of civil society, governments, the scientific and medical communities, and funding providers.

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Abbreviations

The following abbreviations are used in this manuscript:

ARIs	Acute respiratory infections
CT scan	Computed tomography scan
DOAJ	Directory of open access journals
EBV	Epstein–Barr Virus
HRQoL	Health-related quality of life
LCA	Latent class analysis
LD	Linear dichroism
MDPI	Multidisciplinary Digital Publishing Institute
ME/CFS	Myalgic encephalomyelitis/chronic fatigue syndrome
PASC	Post-COVID syndrome
POTS	Postural orthostatic tachycardia syndrome
TLA	Three-letter acronym
WHO	World Health Organization

References

1. Batista, K.B.C.; Fernandez, M.V.; Barberia, L.G.; Silva, E.T.; Pedi, V.D.; Pontes, B.M.L.M.; Araujo, G.; Moreira, R.S.; Pedrosa, M.; Verotti, M.P.; et al. Panorama da COVID longa no Brasil: Análise preliminar de um inquérito para pensar políticas de saúde. *Cad. Saúde Pública* **2024**, *40*, e00094623. [PubMed]
2. Greenhalgh, T.; Knight, M.; A'Court, C.; Buxton, M.; Husain, L. Management of Post-Acute COVID-19 in Primary Care. *BMJ* **2020**, *370*, m3026. [CrossRef] [PubMed]
3. Rodriguez-Sanchez, I.; Rodriguez-Mañas, L.; Laosa, O. Long COVID-19: The Need for an Interdisciplinary Approach. *Clin. Geriatr. Med.* **2022**, *38*, 533–544. [CrossRef] [PubMed]
4. Zavaleta-Monestel, M.; Martínez, A.; Rodríguez, M.; Martínez, J.; Vega, M. Spectrum of Long COVID Symptoms and Management Approaches in Europe and Latin America. *Coronaviruses* **2024**, *1*, 1–15. [CrossRef]
5. Greenhalgh, T.; Sivan, M.; Perlowski, A.; Nikolich, J.Ž. Long COVID: A Clinical Update. *Lancet* **2024**, *404*, 707–724. [CrossRef]
6. Sudre, C.H.; Murray, B.; Varsavsky, T.; Graham, M.S.; Penfold, R.S.; Bowyer, R.C.; Steves, C.J.; Wolf, J.; Spector, T.D.; Ourselin, S. Attributes and Predictors of Long COVID. *Nat. Med.* **2021**, *27*, 626–631. [CrossRef]
7. Nittas, V.; Gao, M.; West, E.A.; Ballouz, T.; Menges, D.; Wulf Hanson, S.; Puhan, M.A. Long COVID Through a Public Health Lens: An Umbrella Review. *Public Health Rev.* **2022**, *43*, 1604501. [CrossRef]
8. Davis, H.E.; Assaf, G.S.; McCorkell, L.; Wei, H.; Low, R.J.; Re'em, Y.; Redfield, S.; Austin, J.P.; Akrami, A. Characterizing Long COVID in an International Cohort: 7 Months of Symptoms and Their Impact. *eClinicalMedicine* **2021**, *38*, 101019. [CrossRef]
9. Al-Aly, Z.; Xie, Y.; Bowe, B. High-Dimensional Characterization of Post-Acute Sequelae of COVID-19. *Nature* **2021**, *594*, 259–264. [CrossRef]
10. de Saboia, T.B.; da Silva, S.F.R. Síndrome da COVID Longa: Uma Revisão de Literatura. *Rev. Multidiscip. Saúde* **2021**, *2*, 181. [CrossRef]
11. Pazukhina, E.; Garcia-Gallo, E.; Reyes, L.F.; Kildal, A.B.; Jassat, W.; Dryden, M.; Holter, J.C.; Chatterjee, A.; Gomez, K.; Søraas, A.; et al. Long COVID: A Global Health Issue—A Prospective, Cohort Study Set in Four Continents. *BMJ Glob. Health* **2024**, *9*, e015245. [CrossRef] [PubMed]
12. Cooper, E.; Lound, A.; Atchison, C.J.; Whitaker, M.; Eccles, C.; Cooke, G.S.; Elliott, P.; Ward, H. Awareness and Perceptions of Long COVID among People in the REACT Programme: Early Insights from a Pilot Interview Study. *PLoS ONE* **2023**, *18*, e0280943. [CrossRef] [PubMed]
13. Evans, R.A.; McAuley, H.J.C.; Harrison, E.M.; Shikotra, A.; Singapuri, A.; Sereno, M.; Elneima, O.; Docherty, A.B.; Lone, N.I.; Leavy, O.C.; et al. Physical, Cognitive, and Mental Health Impacts of COVID-19 after Hospitalisation (PHOSP-COVID): A UK Multicentre, Prospective Cohort Study. *Lancet Respir. Med.* **2021**, *9*, 1275–1287. [CrossRef]

14. Nalbandian, A.; Sehgal, K.; Gupta, A.; Madhavan, M.V.; McGroder, C.; Stevens, J.S.; Cook, J.R.; Nordvig, A.; Shalev, D.; Sehrawat, T.S.; et al. Post-Acute COVID-19 Syndrome. *Nat. Med.* **2021**, *27*, 601–615. [CrossRef]
15. Zaidi, A.K.; Dehgani-Mobaraki, P. Long COVID. *Prog. Mol. Biol. Transl. Sci.* **2024**, *202*, 113–125. [CrossRef]
16. d'Ettorre, G.; Vassalini, P.; Coppolelli, V.; Gentilini Cacciola, E.; Sanitinelli, L.; Maddaloni, L.; Fabris, S.; Mastroianni, C.M.; d'Ettorre, G.; Ceccarelli, G. Health-Related Quality of Life in Survivors of Severe COVID-19 Infection. *Pharmacol. Rep.* **2022**, *74*, 1286–1295. [CrossRef]
17. Aguado, J.; Amarilla, A.A.; Taherian Fard, A.; Albornoz, E.A.; Tyshkovskiy, A.; Schwabenland, M.; Chaggar, H.K.; Modhiran, N.; Gómez-Inclán, C.; Javed, I.; et al. Senolytic Therapy Alleviates Physiological Human Brain Aging and COVID-19 Neuropathology. *Nat. Aging* **2023**, *3*, 1561–1575. [CrossRef]
18. Annukka, A.R.; Antar, A.; Andrea, L.; Cox, L. Translating Insights into Therapies for Long COVID. *Sci. Transl. Med.* **2024**, *16*, eado2106. [CrossRef]
19. Singh, S.J.; Baldwin, M.M.; Daynes, E.; Evans, R.A.; Greening, N.J.; Jenkins, R.G.; Lone, N.I.; McAuley, H.; Mehta, P.; Newman, J.; et al. Respiratory Sequelae of COVID-19: Pulmonary and Extrapulmonary Origins, and Approaches to Clinical Care and Rehabilitation. *Lancet Respir. Med.* **2023**, *11*, 709–725. [CrossRef]
20. Sykes, D.L.; Holdsworth, L.; Jawad, N.; Gunasekera, P.; Morice, A.H.; Crooks, M.G. Post-COVID-19 Symptom Burden: What Is Long-COVID and How Should We Manage It? *Lung* **2021**, *199*, 113–119. [CrossRef]
21. Atchley-Challenner, R.; Strasser, Z.; Krishnamoorthy, A.; Pant, D.; Chibnik, L.B.; Karlson, E.W. Long COVID: A Narrative Review and Meta-Analysis of Individual Symptom Frequencies. *COVID* **2024**, *4*, 1513–1545. [CrossRef]
22. Hirschtick, J.; Slocum, E.; Xie, Y.; Power, L.; Elliott, M.; Orellana, R.; Fleischer, N. Associations Between Acute COVID-19 Symptom Profiles and Long COVID Prevalence: Population-Based Cross-Sectional Study. *JMIR Public Health Surveill.* **2024**, *10*, e55697. [CrossRef] [PubMed]
23. Servier, C.; Porcher, R.; Pane, I.; Ravaud, P.; Tran, V.T. Trajectories of the evolution of post-COVID-19 condition, up to two years after symptoms onset. *Int. J. Infect. Dis.* **2023**, *133*, 67–74. [CrossRef] [PubMed] [PubMed Central]
24. Sharma, S.K.; Mohan, A.; Upadhyay, V. Long COVID syndrome: An unfolding enigma. *Indian J. Med. Res.* **2024**, *159*, 585–600. [CrossRef]
25. Li, J.; Zhou, Y.; Ma, J.; Zhang, Q.; Shao, J.; Liang, S.; Yu, Y.; Li, W.; Wang, C. The long-term health outcomes, pathophysiological mechanisms and multidisciplinary management of long COVID. *Signal Transduct. Target. Ther.* **2023**, *8*, 416. [CrossRef]
26. Pfaff, E.R.; Girvin, A.T.; Bennett, T.D.; Bhatia, A.; Brooks, I.M.; Deer, R.R.; Dekermanjian, J.P.; Jolley, S.E.; Kahn, M.G.; Kostka, K.; et al. Identifying Who Has Long COVID in the USA: A Machine Learning Approach Using N3C Data. *Lancet Digit. Health* **2022**, *4*, e532–e541. [CrossRef]
27. Song, X.; Song, W.; Cui, L.; Duong, T.Q.; Pandy, R.; Liu, H.; Zhou, Q.; Sun, J.; Liu, Y.; Li, T. A Comprehensive Review of the Global Epidemiology, Clinical Management, Socio-Economic Impacts, and National Responses to Long COVID with Future Research Directions. *Diagnostics* **2024**, *14*, 1168. [CrossRef]
28. Wahab, O.A.; Mustafa, A.; Bamatakina, A.B.A. Trends, Politics, Sentiments, and Misinformation: Understanding People's Reactions to COVID-19 During Its Early Stages. *arXiv* **2021**, arXiv:2106.13385. [CrossRef]
29. Madziva, R.; Nachipo, B.; Musuka, G.; Chitungo, I.; Murewanhema, G.; Phiri, B.; Dzinamarira, T. The Role of Social Media during the COVID-19 Pandemic: Salvaging Its 'Power' for Positive Social Behaviour Change in Africa. *Health Promot. Perspect.* **2022**, *12*, 22–27. [CrossRef]
30. Basch, C.H.; Basch, C.E.; Hillyer, G.C.; Meleo-Erwin, Z.C. Social Media, Public Health, and Community Mitigation of COVID-19: Challenges, Risks, and Benefits. *J. Med. Internet Res.* **2022**, *24*, e36804. [CrossRef]
31. Matharaarachchi, S.; Domaratzki, M.; Katz, A.; Muthukumarana, S. Discovering Long COVID Symptom Patterns: Association Rule Mining and Sentiment Analysis in Social Media Tweets. *JMIR Form. Res.* **2022**, *6*, e37984. [CrossRef] [PubMed]
32. Garrett, C.; Qiao, S.; Li, X. The Role of Social Media in Knowledge, Perceptions, and Self-Reported Adherence Toward COVID-19 Prevention Guidelines: Cross-Sectional Study. *JMIR Infodemiology* **2024**, *4*, e44395. [CrossRef] [PubMed]
33. Su, Y.; Fu, H.; Zhang, F.; Deng, T.; Borah, P. Accidently Acquired? Examining the Conditional Indirect Effects of Social Media Incidental News Exposure on Knowledge of COVID-19. *Comput. Hum. Behav.* **2024**, *152*, 108046. [CrossRef]
34. Kenny, G.; Townsend, L.; Savinelli, S.; Mallon, P.W.G. Long COVID: Clinical Characteristics, Proposed Pathogenesis and Potential Therapeutic Targets. *Front. Mol. Biosci.* **2023**, *10*, 1157651. [CrossRef]
35. Perumal, R.; Shunmugam, L.; Naidoo, K.; Abdool Karim, S.S.; Wilkins, D.; Garzino-Demo, A.; Brechot, C.; Parthasarathy, S.; Vahlne, A.; Nikolich, J.Ž. Long COVID: A Review and Proposed Visualization of the Complexity of Long COVID. *Front. Immunol.* **2023**, *14*, 1117464. [CrossRef]
36. Parums, D.V. Long COVID or Post-Acute Sequelae of SARS-CoV-2 Infection (PASC) and the Urgent Need to Identify Diagnostic Biomarkers and Risk Factors. *Med. Sci. Monit.* **2024**, *30*, e946512. [CrossRef]

37. Altmann, D.M.; Whettlock, E.M.; Liu, S.; Arachchillage, D.J.; Boyton, R.J. The Immunology of Long COVID. *Nat. Rev. Immunol.* **2023**, *23*, 618–634. [CrossRef]
38. Research Accessibility Team (RAT). The microvascular hypothesis underlying neurologic manifestations of long COVID-19 and possible therapeutic strategies. *Cardiovasc. Endocrinol. Metab.* **2021**, *10*, 193–203. [CrossRef] [PubMed] [PubMed Central]
39. Camazón, N.V.; Teis, A.; Membrive, M.J.; Llibre, C.; Bayes-Genis, A.; Mateu, L. Long COVID-19 and microvascular disease-related angina. *Rev. Esp. Cardiol. (Engl. Ed.)* **2022**, *75*, 444–446. [CrossRef] [PubMed] [PubMed Central]
40. Liew, F.; Efstathiou, C.; Openshaw, P.J.M. Long COVID: Clues about causes. *Eur. Respir. J.* **2023**, *61*, 2300409. [CrossRef]
41. Peluso, M.J.; Deeks, S.G. Mechanisms of Long COVID and the Path toward Therapeutics. *Cell* **2024**, *187*, 5500–5529. [CrossRef] [PubMed]
42. Chaves, E.C.R.; Quaresma, J.A.S.; Rodrigues, M.H.C.; de Menezes, D.C.; de Lima, I.C.; de Sousa, J.R.; Galúcio, V.C.A.; Queiroz, M.A.F.; Brasil-Costa, I.; Barros, M.C.; et al. Altered Leukocyte Pattern and Inflammatory Markers in Unvaccinated Long COVID Patients: A Cross-Sectional Study. *Sci. Rep.* **2024**, *14*, 28617. [CrossRef] [PubMed]
43. Dhingra, S.; Fu, J.; Cloherty, G.; Mallon, P.; Wasse, H.; Moy, J.; Landay, A.; Kenny, G. Identification of Inflammatory Clusters in Long-COVID through Analysis of Plasma Biomarker Levels. *Front. Immunol.* **2024**, *15*, 1385858. [CrossRef]
44. Rodriguez, L.; Brodin, P. Immune System Perturbations in Patients with Long COVID. *Trends Mol. Med.* **2024**, *30*, 200–201. [CrossRef]
45. Vaivode, K.; Saksis, R.; Litvina, H.D.; Niedra, H.; Sprin, g̀ e, M.L.; Kru¯ min, a, U.; Klovin, š, J.; Rovite, V. Single-Cell RNA Sequencing Reveals Alterations in Patient Immune Cells with Pulmonary Long COVID-19 Complications. *Curr. Issues Mol. Biol.* **2024**, *46*, 461–468. [CrossRef]
46. Ghazanfari, T. Investigating the Immune Dysregulation in Long COVID-19. *Immunoregulation* **2024**, *6*, 79–82. [CrossRef]
47. Molnar, T.; Lehoczki, A.; Fekete, M.; Varnai, R.; Zavori, L.; Erdo-Bonyar, S.; Simon, D.; Berki, T.; Csecsei, P.; Ezer, E. Mitochondrial Dysfunction in Long COVID: Mechanisms, Consequences, and Potential Therapeutic Approaches. *GeroScience* **2024**, *46*, 5267–5286. [CrossRef]
48. Li, S.; Zhao, F.; Ye, J.; Li, K.; Wang, Q.; Du, Z.; Yue, Q.; Wang, S.; Wu, Q.; Chen, H. Cellular Metabolic Basis of Altered Immunity in the Lungs of Patients with COVID-19. *Med. Microbiol. Immunol.* **2022**, *211*, 49–69. [CrossRef]
49. Kovarik, J.J.; Bileck, A.; Hagn, G.; Meier-Menches, S.M.; Frey, T.; Kaempf, A.; Hollenstein, M.; Shoumariyeh, T.; Skos, L.; Reiter, B.; et al. A Multi-Omics Based Anti-Inflammatory Immune Signature Characterizes Long COVID-19 Syndrome. *iScience* **2023**, *26*, 105717. [CrossRef]
50. Huckestein, B.R.; Alcorn, J.F. Improving Mitochondrial Function in Viral Infection: Targeting Cellular Metabolism. *Am. J. Respir. Cell Mol. Biol.* **2022**, *66*, 598–600. [CrossRef]
51. Dempsey, B.; Madan, I.; Stevelink, S.A.M.; Lamb, D. Long COVID among Healthcare Workers: A Narrative Review of Definitions, Prevalence, Symptoms, Risk Factors and Impacts. *Br. Med. Bull.* **2024**, *151*, 16–35. [CrossRef] [PubMed]
52. Da Silva, R.; Vallinoto, A.C.R.; Dos Santos, E.J.M. The Silent Syndrome of Long COVID and Gaps in Scientific Knowledge: A Narrative Review. *Viruses* **2024**, *16*, 1256. [CrossRef] [PubMed]
53. Ashmawy, R.; Hammouda, E.A.; El-Maradny, Y.A.; Aboelsaad, I.; Hussein, M.; Uversky, V.N.; Redwan, E.M. Interplay between Comorbidities and Long COVID: Challenges and Multidisciplinary Approaches. *Biomolecules* **2024**, *14*, 835. [CrossRef] [PubMed]
54. Lippi, G.; Sanchis-Gomar, F.; Henry, B.M. COVID-19 and Its Long-Term Sequelae: What Do We Know in 2023? *Pol. Arch. Intern. Med.* **2023**, *133*, 16402. [CrossRef]
55. Minotti, C.; McKenzie, C.; Dewandel, I.; Bekker, C.; Sturniolo, G.; Doni, D.; Giaquinto, C.; Van Der Zalm, M.M.; Donà, D. How Does Post COVID Differ from Other Post-Viral Conditions in Childhood and Adolescence (0–20 Years Old)? A Systematic Review. *eClinicalMedicine* **2024**, *68*, 102436. [CrossRef]
56. Pintos-Pascual, I.; Moreno-Torres, V.; Ibánez-Estéllez, F.; Corrales-Rodriguez, P.; Treviño, A.; Corpas, M.; Corral, O.; Soriano, V.; De Mendoza, C. Is SARS-CoV-2 the Only Cause of Long-COVID? *AIDS Rev.* **2024**, *24*, 9863. [CrossRef]
57. Chandan, J.S.; Brown, K.R.; Simms-Williams, N.; Bashir, N.Z.; Camaradou, J.; Heining, D.; Turner, G.M.; Rivera, S.C.; Hotham, R.; Minhas, S.; et al. Non-Pharmacological Therapies for Post-Viral Syndromes, Including Long COVID: A Systematic Review. *Int. J. Environ. Res. Public Health* **2023**, *20*, 3477. [CrossRef]
58. Vivaldi, G.; Pfeffer, P.E.; Talaei, M.; Basera, T.J.; Shaheen, S.O.; Martineau, A.R. Long-Term Symptom Profiles after COVID-19 vs Other Acute Respiratory Infections: An Analysis of Data from the COVIDENCE UK Study. *eClinicalMedicine* **2023**, *65*, 102251. [CrossRef]
59. Wang, F. Could We Designate Long COVID a Syndrome? *Sci. Insights* **2024**, *44*, 1389–1391. [CrossRef]
60. Roth, P.H.; Gadebusch-Bondio, M. The Contested Meaning of "Long COVID"—Patients, Doctors, and the Politics of Subjective Evidence. *Soc. Sci. Med.* **2022**, *292*, 114619. [CrossRef]
61. Martínez-Bretón, P.; Colonna-Aguirre, B.; Zebadúa Rodríguez, N.; Iñiguez-Luna, M.I.; Cadena-Zamudio, J.D. *Current Perspectives on Long-COVID: A Brief Review of Understanding and Management*; Agro Productividad: Texcoco, Mexico, 2024. [CrossRef]

62. Van Der Heide, I.; Hansen, J. Mapping Long COVID across the EU: Definitions, Guidelines and Surveillance Systems in EU Member States. *Eur. Public Health Conf.* **2024**, *34*, ckae144.501. [CrossRef]
63. Zeraatkar, D.; Ling, M.; Kirsh, S.; Jassal, T.; Shahab, M.; Movahed, H.; Talukdar, J.R.; Walch, A.; Chakraborty, S.; Turner, T.; et al. Interventions for the management of long COVID (post-COVID condition): Living systematic review. *BMJ* **2024**, *387*, e081318. [CrossRef] [PubMed] [PubMed Central]

3. DISCUSSÃO INTEGRADA AMPLIADA

A pandemia de COVID-19 representou um marco histórico para a compreensão das infecções respiratórias, não apenas pelo impacto direto do SARS-CoV-2, mas também pelas transformações subsequentes na dinâmica viral, nos padrões clínicos e nos sistemas de saúde. A análise conjunta dos três capítulos desta tese — abrangendo a circulação viral durante a pandemia, a reorganização pós- pandêmica e a COVID longa — revela que a crise sanitária não foi um evento isolado, mas um processo de reorganização ecológica, social e clínica de longo prazo (Eden et al., 2022; Greenhalgh et al., 2024).

O SARS-CoV-2 atuou como um agente disruptivo, remodelando a cocirculação viral e impondo novas pressões seletivas sobre outros patógenos respiratórios. Inicialmente, o predomínio quase absoluto da COVID-19 reduziu drasticamente a detecção de vírus como Influenza e RSV, mas não eliminou totalmente sua circulação (Peci et al., 2021; Eden et al., 2022). O HRV, por exemplo, manteve atividade significativa, sugerindo que determinados vírus possuem resiliência ecológica frente a intervenções sociais e imunológicas.

Esse cenário se desdobrou em uma reorganização no período pós-pandêmico, quando RSV e Influenza retornaram com padrões sazonais alterados e intensidade incomum. O conceito de débito imunológico ganhou relevância nesse contexto. Durante quase dois anos, as medidas de controle não farmacológicas reduziram drasticamente a circulação de vírus respiratórios como RSV e Influenza (Cohen et al., 2021; Hatter et al., 2021). Essa redução, embora benéfica no curto prazo, interrompeu o ciclo natural de exposição e reforço imunológico em crianças e adultos jovens.

Esse fenômeno se refletiu especialmente em recém-nascidos e lactentes. Muitas mães não tiveram infecções respiratórias durante a gestação e, portanto, transferiram menos anticorpos protetores por via transplacentária (Bardsley et al., 2022). Isso pode ter contribuído para hospitalizações mais graves em lactentes, sugerindo que o débito imunológico não afeta apenas indivíduos, mas também a transmissão vertical de imunidade entre gerações. Além disso, o hiato de exposição também pode ter reduzido a memória imunológica em crianças mais velhas, ampliando a coorte suscetível.

O rebote epidemiológico complementa esse quadro, destacando o papel das mudanças sociais abruptas. Ao suspender rapidamente as medidas de contenção como fechamento de escolas, uso de máscaras e restrição de mobilidade criou-se uma condição ideal para surtos explosivos (CIDRAP, 2022). Não se tratou apenas de aumento gradual de suscetíveis, mas de uma liberação súbita do contato social em populações imunologicamente vulneráveis.

Esse fenômeno foi observado em diversos países, com surtos de RSV e Influenza fora de época, desafiando modelos tradicionais de sazonalidade (Eden et al., 2022; Liu & Rocklöv, 2022). Uma hipótese é que o rebote epidemiológico seja ainda mais intenso em ambientes urbanos, onde densidade populacional e mobilidade aceleram a transmissão, favorecendo surtos explosivos em populações vulneráveis (Rocklöv & Sjödin, 2020). Dessa forma, o rebote não apenas reorganizou a circulação viral, mas também acentuou desigualdades territoriais, com impacto desproporcional em grandes centros urbanos e comunidades vulneráveis.

Outro elemento fundamental é a interferência viral, um fenômeno ecológico em que a circulação de um vírus pode inibir ou modular a de outro, seja por mecanismos imunológicos (como indução de interferons) ou por competição direta por hospedeiros suscetíveis. Durante a pandemia, o predomínio do SARS-CoV-2 coincidiu com a redução drástica de Influenza e RSV, levantando a hipótese de que parte desse efeito não se deveu apenas às medidas sociais, mas também à supressão mediada pelo próprio SARS-CoV-2 (Faria et al., 2021; Sabino et al., 2021).

A circulação predominante da variante Gama (P.1) em 2021 esteve associada a uma maior gravidade clínica da COVID-19, em comparação a linhagens anteriores. Estudos apontaram que a Gama, identificada inicialmente em Manaus no final de 2020, foi responsável por um rápido aumento de hospitalizações e mortalidade, incluindo em faixas etárias mais jovens do que observado nas ondas iniciais (Faria et al., 2021; Sabino et al., 2021). Essa maior severidade pode estar relacionada a mutações específicas na proteína spike, como E484K, K417T e N501Y, que aumentaram a afinidade pelo receptor ACE2 e conferiram escape parcial da imunidade adquirida em infecções prévias ou após vacinação incompleta (Nonaka et al., 2021; Souza et al., 2021). No contexto brasileiro, marcado por alta transmissão comunitária e desigualdades no acesso à saúde, a disseminação da Gama levou a uma sobrecarga hospitalar sem precedentes, mascarando a real circulação de outros vírus respiratórios naquele período. Esses achados reforçam a necessidade de interpretar a dinâmica viral não apenas sob a ótica da cocirculação, mas também considerando o

papel central das variantes na remodelação do perfil clínico-epidemiológico das infecções respiratórias.

No período pós-pandêmico, observou-se outro padrão: em meses de maior circulação de RSV, a detecção de SARS-CoV-2 diminuiu, sugerindo um possível efeito de exclusão competitiva (Powell et al., 2023). A interferência viral não é nova e já foi descrita entre Influenza e rinovírus em epidemias anteriores (Eames et al., 2023), mas a pandemia trouxe uma oportunidade única de observá-la em escala global. A implicação é que a ecologia viral respiratória é regulada por interações dinâmicas entre vírus, e que futuros modelos preditivos precisam incorporar essas interações.

Durante o período pós-pandêmico, a circulação do vírus Influenza apresentou picos sazonais inesperados, inclusive fora de época, em contraste com o padrão tradicional observado antes da COVID-19. Esse comportamento atípico tem sido relatado em diversos países e pode estar relacionado ao impacto das medidas de contenção durante a pandemia, que reduziram drasticamente a circulação viral e criaram um acúmulo de suscetíveis. A suspensão das medidas de distanciamento social, somada à baixa exposição prévia, contribuiu para epidemias mais intensas e em períodos incomuns (Olsen et al., 2021; Hirve et al., 2022). Esses achados reforçam a hipótese de que a pandemia funcionou como um "reset imunológico" e ecológico, alterando temporariamente a previsibilidade da sazonalidade da Influenza e trazendo implicações para a vigilância e para a formulação de vacinas sazonais.

A análise integrada mostra que os impactos clínicos foram desiguais entre diferentes grupos. Crianças mais novas sofreram com hospitalizações por RSV, idosos apresentaram maior gravidade em infecções por Influenza e HRV, e indivíduos com comorbidades foram mais suscetíveis a complicações da COVID-19 (Hung et al., 2017; González et al., 2024). Esses perfis reforçam que a vulnerabilidade clínica é multifatorial, determinada pela interação entre idade, estado imunológico, comorbidades e determinantes sociais.

A pandemia também expôs desigualdades estruturais. O acesso desigual ao diagnóstico molecular significou que muitas populações, especialmente em áreas rurais ou em países de baixa renda, apresentaram subnotificação sistemática de infecções respiratórias. Além disso, a distribuição desigual de vacinas exacerbou as diferenças de risco entre grupos e regiões (Evans et al., 2021; Cooper et al., 2023). Essas desigualdades não são periféricas: constituem parte central

da explicação para a variabilidade dos impactos observados.

Outro aspecto crítico é o papel do ambiente urbano como amplificador da transmissão viral. Densidade populacional, transporte público e maior mobilidade social criaram condições para rápida disseminação, enquanto áreas rurais sofreram com subdiagnóstico e atraso na detecção de surtos. Assim, a pandemia reforça a necessidade de interpretar dados epidemiológicos sempre à luz do contexto social e geográfico (Monod et al., 2021).

A elevada proporção de amostras de SARI sem etiologia definida, mesmo após testagem para os principais vírus respiratórios chama a atenção. Esse resultado evidencia lacunas diagnósticas e reforça a necessidade de ampliar os painéis moleculares para incluir outros patógenos relevantes, como metapneumovírus, adenovírus e vírus parainfluenza, frequentemente associados a surtos e hospitalizações, mas subdetectados em rotinas diagnósticas restritas (Tang et al., 2017; Gilca et al., 2014). A inclusão desses vírus na vigilância é crucial não apenas para reduzir a subnotificação, mas também para fornecer um quadro mais completo da dinâmica das infecções respiratórias, permitindo melhores respostas clínicas e de saúde pública.

Se o impacto agudo da pandemia foi evidente, sua expressão crônica é ainda mais desafiadora. A COVID longa emergiu como um novo paradigma de doença pós-infecciosa, caracterizada por diversidade de sintomas, imprevisibilidade e ausência de biomarcadores específicos (Sudre et al., 2021; Davis et al., 2021). A condição rompeu a lógica tradicional da infectologia, ao se apresentar como síndrome crônica de origem infecciosa, afetando múltiplos sistemas e exigindo abordagem interdisciplinar.

Hipóteses fisiopatológicas incluem persistência viral em reservatórios, autoimunidade desencadeada pela infecção, inflamação sustentada e disfunção mitocondrial (Altmann et al., 2023; Molnar et al., 2024). É plausível que múltiplos mecanismos atuem simultaneamente, resultando em fenótipos distintos entre pacientes. Essa heterogeneidade desafia tanto a prática clínica quanto o desenho de ensaios clínicos, que precisam considerar subgrupos específicos de manifestações (Chaves et al., 2024; Rodriguez & Brodin, 2024).

Além da biologia, a COVID longa é também um fenômeno social. A luta pelo reconhecimento clínico, o estigma associado à cronicidade e a infodemia nas redes sociais moldaram a forma como a condição foi percebida por pacientes, médicos e gestores (Roth &

Gadebusch-Bondio, 2022; Garrett et al., 2024). Isso sugere que o legado da pandemia não é apenas biomédico, mas também político e cultural, trazendo à tona condições pós-virais historicamente negligenciadas, como a síndrome da fadiga crônica.

Um dos elementos centrais que marcam a COVID longa é sua condição de "invisibilidade". Diversos relatos apontam que pacientes com sintomas persistentes foram inicialmente desacreditados por profissionais de saúde, sendo suas queixas atribuídas a fatores psicológicos ou secundários à pandemia (Roth & Gadebusch-Bondio, 2022; Cooper et al., 2023). Essa ausência de reconhecimento clínico não apenas atrasou o diagnóstico e a busca por cuidados, mas também gerou estigmatização e sofrimento adicional, ampliando a carga da síndrome. No Brasil, o despreparo do Sistema Único de Saúde (SUS) para identificar e manejar a COVID longa tornou o cenário ainda mais desafiador, uma vez que não existem protocolos clínicos consolidados ou fluxos assistenciais estruturados para lidar com pacientes que apresentam manifestações crônicas pós-COVID. Essa lacuna institucional contribui para a perpetuação da invisibilidade, reforçando a necessidade urgente de capacitação profissional, elaboração de diretrizes nacionais e fortalecimento da vigilância em saúde para reconhecer e dar visibilidade a essa nova síndrome.

A experiência da pandemia e do período subsequente mostra que os sistemas de vigilância precisam ser integrados, flexíveis e abrangentes. Limitar a detecção a poucos vírus não é suficiente: é necessário adotar painéis mais amplos, incorporar metodologias genômicas e considerar variáveis sociais e ambientais nos modelos preditivos (Tang et al., 2017).

A vacinação se mantém como pilar central da prevenção, mas a pandemia demonstrou que campanhas tradicionais precisam ser repensadas. A inclusão de novas vacinas como as contra RSV e a atualização contínua das vacinas de Influenza e SARS-CoV-2 são estratégias fundamentais para mitigar hospitalizações em grupos vulneráveis. Além disso, programas de vacinação devem ser desenhados levando em conta barreiras sociais e territoriais, sob risco de perpetuar desigualdades (Greenhalgh et al., 2024).

Outro aprendizado é a necessidade de descentralizar a capacidade diagnóstica. Durante a pandemia, a concentração de recursos em grandes centros atrasou a resposta em áreas periféricas. Uma vigilância efetiva exige capilaridade laboratorial, treinamento de equipes locais e integração com redes nacionais e internacionais de monitoramento.

Outra lacuna importante é a falta de integração entre vigilância epidemiológica e dados ambientais. A incorporação de variáveis climáticas, padrões de mobilidade e determinantes sociais pode aprimorar a capacidade de prever epidemias sazonais (Gilca et al., 2014). Além disso, há necessidade de fortalecer pesquisas translacionais que conectem achados de bancada com práticas clínicas e políticas públicas.

Finalmente, a pandemia mostrou que epidemias não se encerram quando os casos agudos diminuem. Elas deixam legados de longo prazo, desde reorganizações da circulação viral até condições crônicas como a COVID longa. Preparar-se para o futuro significa reconhecer que pandemias produzem ondas sucessivas: a primeira, de doença aguda; a segunda, de reorganização epidemiológica; e a terceira, de doenças crônicas e desigualdades sociais.

4 CONSIDERAÇÕES FINAIS

Como um todo, as análises apresentadas nos três artigos permitem sugerir inferências, de forma integrada, sobre os perfis epidemiológicos, clínicos e de hospitalização das infecções respiratórias virais em Minas Gerais em diferentes fases da pandemia, além de aprofundar a discussão sobre a COVID longa e suas implicações.

No primeiro recorte, observou-se a predominância da variante Gama do SARS-CoV-2 em 2021, associada a maior gravidade clínica, enquanto Influenza e RSV apresentaram circulação reduzida, evidenciando o impacto direto das medidas de contenção e reforçando a importância da vigilância integrada para orientar decisões em saúde pública. No segundo recorte, em contexto pós-pandêmico, constatou-se o ressurgimento do RSV e da Influenza em sazonalidades atípicas, a circulação contínua do rinovírus e a presença expressiva de casos sem etiologia definida, o que evidenciou lacunas diagnósticas ainda persistentes e a necessidade de ampliar o uso de painéis moleculares para garantir maior precisão na detecção de agentes respiratórios. Finalmente, ao tratar da COVID longa, ficou evidente que se trata de uma condição multissistêmica, de difícil reconhecimento clínico e frequentemente invisibilizada nos serviços de saúde, marcada pela ausência de critérios diagnósticos padronizados e pela carência de políticas específicas que assegurem acompanhamento e cuidado adequados.

Em síntese, esses achados permitem evidenciar como a pandemia de COVID-19 não apenas alterou a dinâmica de circulação dos vírus respiratórios, mas também expôs fragilidades nos sistemas de vigilância e diagnóstico e trouxe à tona um novo desafio relacionado às sequelas pós-infecciosas. À luz da microbiologia e da vigilância epidemiológica, esta tese evidencia que a COVID-19 representou um marco de ruptura na ecologia viral, com efeitos duradouros sobre as síndromes respiratórias agudas graves e sobre a forma como a comunidade científica e os serviços de saúde precisam interpretar e enfrentar as consequências de longo prazo deixadas pela infecção.

A cocirculação principalmente dos vírus Influenza A/B, e RSV no período pós-pandêmico mostrou-se alterada em relação ao padrão sazonal clássico, revelando sobreposição de epidemias e circulação contínua de alguns vírus, como o HRV. Esses achados reforçam a importância da vigilância integrada, apoiada em diagnóstico molecular, para orientar a prática clínica e as políticas públicas de saúde.

O RSV destacou-se em lactentes e crianças pequenas, associado às maiores taxas de hospitalização e UTI, enquanto Influenza A/B e SARS-CoV-2 predominaram em idosos, com evolução clínica também marcada por gravidade. O HRV circulou durante todo o ano e foi o

único vírus cuja carga viral apresentou associação com hospitalização, indicando relevância em populações vulneráveis mesmo sendo considerado, em geral, de evolução branda.

A variante Gama do SARS-CoV-2 apresentou maior gravidade clínica em comparação a outras variantes, especialmente em pacientes com comorbidades como diabetes, obesidade e cardiopatias. Coinfecções virais foram pouco frequentes e não mostraram associação com aumento da gravidade, sugerindo que a evolução clínica esteve mais relacionada ao agente predominante e às condições do hospedeiro.

Uma parcela expressiva dos casos de síndrome respiratória aguda grave (SARI) permaneceu sem etiologia definida mesmo após RT-qPCR para os principais vírus respiratórios, evidenciando limitações diagnósticas atuais e a necessidade de ampliar os painéis laboratoriais para outros vírus e agentes atípicos. Esse achado indica que parte importante do impacto clínico ainda não é capturada pelos sistemas de vigilância tradicionais.

A COVID longa consolidou-se como condição emergente e incapacitante, afetando de forma significativa a qualidade de vida de indivíduos acometidos. Sendo caracterizada por sintomas persistentes e heterogêneos, incluindo fadiga, dispneia e déficit cognitivo, com evidências de envolvimento multissistêmico. Hipóteses apontam para alterações imunológicas, inflamação persistente, estresse oxidativo e disfunção mitocondrial como mecanismos prováveis, mas ainda pouco compreendidos, reforçando a necessidade de estratégias clínicas e políticas públicas específicas para enfrentamento da síndrome.

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5 CONCLUSÃO

- A sazonalidade clássica foi rompida no pós-pandemia e a gravidade distribuiu-se de modo etário-específico. Esse novo padrão de circulação redefine a vigilância e o cuidado justificando a vigilância integrada com diagnóstico molecular ampliado.
- Persistem lacunas diagnósticas nos casos de SARI, indicando a necessidade de expansão de painéis moleculares.
- No eixo crônico, a COVID longa impõe carga funcional e social que requer linhas de cuidado e políticas específicas.
- A variante Gama associou-se a maior gravidade, sobretudo em pacientes portadores de comorbidades. A implicação prática sugere que protocolos de risco devem ponderar o agente predominante em conjunto com o perfil do hospedeiro, resultando em melhor alocação de recursos financeiros e humanos.
- Houve reconfiguração sazonal (Influenza A/B e RSV) com o RSV concentrando casos graves em lactentes/crianças, e Influenza A/B/SARS-CoV-2 predominaram em idosos. O HRV apresentou circulação ao longo do ano e associação com hospitalização. Desta forma a utilização de métodos moleculares ampliados e monitoramento integrado de outros vírus pode tornar a vigilância sentinela mais robusta com resultados aplicáveis aos pacientes.
- A COVID longa é persistente, heterogênea e incapacitante, com provável base multissistêmica. É urgente a necessidade de instituir linhas de cuidado e reabilitação, fluxos de encaminhamento interprofissional e monitoramento longitudinal. Desta discussão surge uma pergunta: quais biomarcadores e perfis clínico-laboratoriais melhor predizem resposta a reabilitação e risco de cronificação?

6. REFERÊNCIAS

1. ALTMANN, D. M.; WHETTLOCK, E. M.; LIU, S.; BOYTON, R. J. The immunology of long COVID. *Nature Reviews Immunology*, v. 23, n. 9, p. 618-634, 2023.

2. BAMBRA, C.; RIORDAN, R.; FORD, J.; MATTHEWS, F. The COVID-19 pandemic and health inequalities. *Journal of Epidemiology & Community Health*, London, v. 74, n. 11, p. 964-968, nov. 2020. DOI: https://doi.org/10.1136/jech-2020-214401.

3. BARDSLEY, M.; MORBEY, R. A.; HUGHES, H. E.; ELLIOT, A. J.; HARCOURT, S.; SMITH, G. E. *et al.* Epidemiology of off-season RSV epidemics in Europe.
Eurosurveillance, v. 27, n. 15, p. 2101110, 2022.

4. BAMBRA, C.; RIORDAN, R.; FORDER, J.; WICKHAM, S. The COVID-19 pandemic and health inequalities. *Journal of Epidemiology and Community Health*, v. 74, n. 11, p. 964–968, nov. 2020. DOI: https://doi.org/10.1136/jech-2020-214401.

5. BOUFIDOU, F. et al. SARS-CoV-2 reinfections and long COVID in the post-Omicron phase of the pandemic. International Journal of Molecular Sciences, v. 24, n. 16, p. 12962, 19 ago. 2023.

6. BRASIL. Ministério da Saúde. Nota técnica n.º 057/2023-DGIP/SE/MS. Brasília, DF: Ministério da Saúde, 2021. Disponível em: https://bvsms.saude.gov.br/bvs/publicacoes/nota_tecnica_n57_atualizacoes_condicoes_pos covid.pdf. Acesso em: 10 maio. 2024.

7. BYAMBASUREN, O. et al. Effect of covid-19 vaccination on long covid: systematic review. BMJ Medicine, v. 2, n. 1, p. e000385, fev. 2023.

8. CASTRO, M. C.; KIM, S.; BARBERIA, L.; RIBEIRO, A. F.; GURZENDA, S.; RIBEIRO, K. B.; ABBOTT, E.; BLOSSOM, J.; RACHE, B.; SINGER, B. H.
Spatiotemporal pattern of COVID-19 spread in Brazil. *Science*, v. 372, n. 6544, p. 821– 826, 21 maio 2021. DOI: https://doi.org/10.1126/science.abh1558.

9. CHAVES, E. C. R.; QUARESMA, J. A. S.; RODRIGUES, M. H. C.; MENEZES, D. C.; LIMA, I. C.; SOUSA, J. R. *et al.* Altered leukocyte pattern and inflammatory markers in long COVID patients. *Scientific Reports*, v. 14, p. 28617, 2024.

10. CHOW, E. J. *et al.* The effects of the COVID-19 pandemic on community respiratory virus activity. *Nature Reviews Microbiology*, 2022.

11. CIDRAP. Immunologic study shows rebound of other viruses after COVID measures lifted. *CIDRAP News*, 2022. Disponível em: https://www.cidrap.umn.edu. Acesso em: 26 ago. 2025.

12. COHEN, R.; ASHMAN, M.; TAHA, M. K.; VARON, E.; ANGOULVANT, F.; LEVY, C.

et al. Pediatric infectious disease group (GPIP) position paper on the immune debt of the COVID-19 pandemic. *Infectious Diseases Now*, v. 51, n. 5, p. 418-423, 2021.

13. COOPER, E.; LOUND, A.; ATCHISON, C. J.; WHITAKER, M.; COOKE, G. S.; ELLIOTT, P. *et al.* Awareness and perceptions of long COVID: insights from the REACT Programme. *PLoS One*, v. 18, n. 2, e0280943, 2023.

14. COSTANZA, G.; PABA, P.; CIOTTI, M.; OMBRES, D.; DI CARLO, S.; MARCUCCILLI, F.; BERTOLI, A.; DI TRAGLIA, L.; MOZZANI, M.; PIRELLA, L. *et al.* Infection rate of respiratory viruses in the pandemic SARS-CoV-2 period considering symptomatic patients: two years of ongoing observations. *Biomolecules*, v. 12, n. 987, 2022. Disponível em: https://doi.org/10.3390/biom12070987. Acesso em: 26 set. 2025.

15. CRODA, J. et al. COVID-19 in Brazil: advantages of a socialized unified health system and preparation to contain cases. *Revista da Sociedade Brasileira de Medicina Tropical*, v. 53, p. e20200167, 2020. DOI: https://doi.org/10.1590/0037-8682-0167-2020.

16. DAVIS, H. E.; ASSAF, G. S.; MCCORKELL, L.; WEI, H.; LOW, R. J.; RE'EM, Y. *et al.* Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *eClinicalMedicine*, v. 38, 101019, 2021.

17. EAMES, K.; TILSTON, N.; BROOKS-POLLOCK, E.; EDMUNDS, W. J. Symptom profiles of community cases infected by influenza, RSV and SARS-CoV-2. *Scientific Reports*, v. 13, p. 13934, 2023.

18. EDEN, J. S.; SIKAZWE, C.; XIE, R.; DENG, Y. M.; SULLIVAN, S. G.; MICHIE, A. *et al.* Off-season RSV epidemics in Australia after easing of COVID-19 restrictions. *Nature Communications*, v. 13, n. 1, p. 2884, 2022.

19. EVANS, R. A.; MCAULEY, H. J. C.; HARRISON, E. M.; SHIKOTRA, A.; SINGAPURI, A.; SERENO, M. *et al.* Physical, cognitive and mental health impacts of COVID-19 after hospitalisation (PHOSP-COVID). *Lancet Respiratory Medicine*, v. 9, n. 12, p. 1275-1287, 2021.

20. FARIA, N. R.; MELLAN, T. A.; WHITTAKER, C.; CLARO, I. M.; CANDIDO, D. S.; MISHRA, S. *et al.* Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*, v. 372, n. 6544, p. 815-821, 2021.

21. GARRETT, C.; QIAO, S.; LI, X. Role of social media in perceptions and adherence toward COVID-19 prevention. *JMIR Infodemiology*, v. 4, e44395, 2024.

22. GILCA, R.; AMINI, R.; DOUVILLE-FRADET, M.; CHAREST, H.; DUBUQUE, J.; BOULIANNE, N. *et al.* Other respiratory viruses are important contributors to adult respiratory hospitalizations and mortality even during peak weeks of the influenza season. *Open Forum Infectious Diseases*, v. 1, n. 2, ofu086, 2014.

23. GONZÁLEZ, R.; DÍAZ, L.; CALDERÓN, M. E. *et al.* Factors associated with outcome in national cohort of rhinovirus infection in adults. *Scientific Reports*, v. 14, p. 2458, 2024.

24. GREENHALGH, T.; SIVAN, M.; PERLOWSKI, A.; NIKOLICH, J. Ž. Long COVID: a

clinical update. *Lancet*, v. 404, n. 10318, p. 707-724, 2024.

25. HANAGE, W. P.; SCHAFFNER, W. Burden of acute respiratory infections caused by influenza virus, respiratory syncytial virus, and SARS-CoV-2 with consideration of older adults: a narrative review. *Infectious Diseases and Therapy*, v. 14, n. S1, p. 5–37, jan. 2025.

26. HATTER, L.; EATHORNE, A.; HILLS, T.; BRUCE, P.; BEASLEY, R. Respiratory syncytial virus: paying the immunity debt with interest. *Lancet Child & Adolescent Health*, v. 5, n. 12, p. e44–e45, 2021.

27. HIRVE, S.; NEWMAN, L. P.; PAGET, J.; AZZIZ-BAUMGARTNER, E.; FITZNER, J.; BHAT, N. Influenza seasonality in the post-COVID-19 era: what can we expect? *Influenza and Other Respiratory Viruses*, v. 16, n. 5, p. 926–936, 2022.

28. HUNG, I. F. N.; ZHANG, A. J.; TO, K. K. W. *et al.* High morbidity and mortality in adults hospitalized for rhinovirus infections. *Respiratory Research*, v. 18, n. 1, p. 11, 2017.

29. KAMPENUSA, I.; *et al.* Circulation and codetections of influenza virus, SARS-CoV-2, respiratory syncytial virus, rhinovirus, adenovirus, bocavirus, and other respiratory viruses during 2022–2023 season in Latvia. *Viruses*, 2024.

30. KRASHIAS, G.; *et al.* Prevalence of viral and bacterial co-infections in SARS-CoV-2-positive individuals in Cyprus 2020–2022. *Biomedicines*, 2025.

31. LIU, Y.; ROCKLÖV, J. The reproductive number of seasonal influenza and its implications. *International Journal of Environmental Research and Public Health*, v. 19, n. 1, p. 123, 2022.

32. MA, S. et al. Metagenomic analysis reveals oropharyngeal microbiota alterations in patients with COVID-19. Signal Transduction and Targeted Therapy, v. 6, n. 1, p. 191, 13 maio 2021.

33. MAGALHÃES BRONZE, K.; DOS SANTOS, U. R.; BARBOSA COSTA, G.; SEVÁ, A. P.; GUIMARÃES KERSUL, M.; SACRAMENTO PINTO, C.; REGO ALBUQUERQUE, G.; MELO MARIANO, A. P.; GADELHA, S. R. The impact of the COVID-19 pandemic on the clinical and epidemiological profile of severe acute respiratory infection in Bahia, Brazil: a comparative analysis of pre- and post-pandemic trends. *Viruses*, v. 17, p. 389, 2025. DOI: https://doi.org/10.3390/v17030389.

34. MARTINÓN-TORRES F, Gutierrez C, Cáceres A, Weber K, Torres A. How Does the Burden of Respiratory Syncytial Virus Compare to Influenza in Spanish Adults?. *Influenza Other Respir Viruses.* 2024;18(6):e13341. doi:10.1111/irv.13341

35. MANIRAMBONA, E.; OKESANYA, O. J.; OLALOKE, N.; OSO, T. A.; LUCERO-PRISNO III, D. E. Evolution and implications of SARS-CoV-2 variants in the post- pandemic era. *Discover Public Health*, v. 21, artigo 16, 28 jun. 2024. Disponível em: https://link.springer.com/article/10.1186/s12982-024-00140-x. Acesso em: 20 julho. 2025

36. MARRA, A. R. et al. Risk factors for long COVID among healthcare workers, Brazil, 2020–2022. medRxiv, 5 jan. 2023. Disponível em:

http://medrxiv.org/lookup/doi/10.1101/2023.01.03.22284043. Acesso em: 27 jul. 2024.

37. MOJICA-CRESPO, R.; MORALES-CRESPO, M. M. Pandemia COVID-19, la nueva emergencia sanitaria de preocupación internacional: una revisión. *Medicina de Familia. SEMERGEN*, v. 46, p. 65–77, ago. 2020. DOI: https://doi.org/10.1016/j.semerg.2020.05.010.

38. MOLNAR, T.; LEHOCZKI, A.; FEKETE, M.; VARNAI, R.; ZAVORI, L.; ERDO-BONYAR, S. *et al.* Mitochondrial dysfunction in long COVID: mechanisms and therapeutic approaches. *GeroScience*, v. 46, n. 2, p. 5267–5286, 2024.

39. MONOD, M.; BLENKINSOP, A.; XI, X.; HEBERT, D.; BERSHAN, S.; TIETZE, S. *et al.* Age groups that sustain resurging COVID-19 epidemics in the US. *Science*, v. 371, n. 6536, eabe8372, 2021.

40. MOSTAFA, H. H.; *et al.* Respiratory virus disease and outcomes at a large academic medical center in the United States: a retrospective observational study of the early 2023/2024 respiratory viral season. *Microbiology Spectrum*, v. 12, n. 10, 3 out. 2024.

41. NONAKA, C. K. V.; FRANCO, M. M.; GRÄF, T.; BARCIA, C. A. L.; MENDONÇA, R. M. R.; DE SOUSA, K. A. F.; *et al.* Genomic evidence of SARS-CoV-2 reinfection involving E484K spike mutation, Brazil. *Emerging Infectious Diseases*, v. 27, n. 5, p. 1522–1524, 2021.

42. OLSEN, S. J.; WINN, A. K.; BUDD, A. P.; PRILL, M. M.; STEEL, J.; MIDGLEY, C. M.; *et al.* Changes in influenza and other respiratory virus activity during the COVID-19 pandemic — United States, 2020–2021. *MMWR Morbidity and Mortality Weekly Report*, v. 70, n. 29, p. 1013–1019, 2021.

43. PAPAGIANNIS, D.; *et al.* Proportion of respiratory syncytial virus, SARS-CoV-2, influenza A/B, and adenovirus cases via rapid tests in the community during winter 2023—a cross-sectional study. *Diseases*, v. 11, n. 3, p. 122, 15 set. 2023.

44. PARUMS, D. V. Long COVID and the urgent need to identify diagnostic biomarkers and risk factors. *Medical Science Monitor*, v. 30, e946512, 2024.

45. PECI, A.; TRAN, V.; GUTHRIE, J. L.; GUBBAY, J. B. Impact of public health measures on the 2020 RSV and influenza season in Canada. *Clinical Infectious Diseases*, v. 73, n. 6, p. e1406–e1409, 2021.

46. POWELL, A. A.; NOLAN, M.; KEMPPAINEN, L.; BLOMQVIST, S.; NOHYNEK, H.; LYYTIKÄINEN, O.; *et al.* Symptom profiles during co-circulation of SARS-CoV-2, influenza and RSV in England. *BMC Medicine*, v. 21, p. 170, 2023.

47. ROCKLÖV, J.; SJÖDIN, H. High population densities catalyze the spread of COVID-19. *Journal of Travel Medicine*, v. 27, n. 3, taaa038, 2020.

48. RODRIGUEZ, L.; BRODIN, P. Immune system perturbations in patients with long COVID. *Trends in Molecular Medicine*, v. 30, n. 3, p. 200–201, 2024.

49. ROTH, P. H.; GADEBUSCH-BONDIO, M. The contested meaning of “long COVID”:

patients, doctors, and politics of subjective evidence. *Social Science & Medicine*, v. 292, 114619, 2022.

50. ROTH, P. H.; GADEBUSCH-BONDIO, M. The contested meaning of "long COVID": patients, doctors, and the politics of subjective evidence. *Social Science & Medicine*, v. 292, p. 114619, 2022.

51. SABINO, E. C.; BUSS, L. F.; CARVALHO, M. P. S.; PRETE, C. A. Jr; CRISPIM, M. A. E.; FRAIJI, N. A.; *et al.* Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. *The Lancet*, v. 397, n. 10273, p. 452–455, 2021.

52. SAPRA, M.; *et al.* Respiratory viral infections other than SARS-CoV-2 among the North Indian patients presenting with acute respiratory illness during the first COVID-19 wave. *Virus Disease*, 2022.

53. SBERNA, G.; *et al.* A retrospective study of respiratory viruses in a four-year study of nasal swabs from patients with severe influenza-like symptoms in the Lazio region, Italy. *Viruses*, 2025.

54. SIQUEIRA, B. A.; *et al.* Viral co-detection of influenza virus and other respiratory viruses in hospitalized Brazilian patients during the first three years of the coronavirus disease (COVID)-19 pandemic: an epidemiological profile. *Frontiers in Microbiology*, 2024.

55. SOARES, T. D. N.; *et al.* Risk of incidence and lethality by etiology of severe acute respiratory syndrome in hospitalized children under 1 year of age in Brazil in 2024: a cross-sectional study. *Tropical Medicine and Infectious Disease*, 2025.

56. SOUZA, W. M.; AMORIM, M. R.; SESTI-COSTA, R.; COIMBRA, L. D.; BRUNETTI, N. S.; TOLEDO-TEIXEIRA, D. A.; *et al.* Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination. *The Lancet Microbe*, v. 2, n. 10, p. e527–e535, 2021.

57. SUDRE, C. H.; MURRAY, B.; VARSAVSKY, T.; GRAHAM, M. S.; PENFOLD, R. S.; BOWYER, R. C.; *et al.* Attributes and predictors of long COVID. *Nature Medicine*, v. 27, n. 4, p. 626–631, 2021.

58. TANG, J. W.; LAM, T. T.; ZARAKET, H.; LIPKIN, W. I.; DREWS, S. J.; HATCHETTE, T. F.; *et al.* Global epidemiology of non-influenza RNA respiratory viruses: data gaps and a growing need for surveillance. *The Lancet Infectious Diseases*, v. 17, n. 10, p. e320– e326, 2017.

59. TRIFONOVA I.; *et al.* Epidemiological and Genetic Characteristics of Respiratory Viral Coinfections with Different Variants of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Viruses.* 2024;16(6):958. Published 2024 Jun 13. doi:10.3390/v16060958

60. VIANNA, L. A.; *et al.* Seasonality, molecular epidemiology, and virulence of respiratory syncytial virus (RSV): a perspective into the Brazilian Influenza Surveillance Program. *PLOS ONE*, 2021.

61. WATANABE, A.; *et al.* Viral etiology among the elderly presenting acute respiratory infection during the influenza season / Etiologia viral de infecção respiratória aguda entre idosos, durante a temporada de gripe. *Revista da Sociedade Brasileira de Medicina Tropical*, 2011.

62. YOU, L.; ZHANG, Z.; LI, H.; WENG, Y.; CALDER, P. Exploring the respiratory viral landscape beyond influenza and SARS-CoV-2: a 2020–2023 study in Fujian, China. *Frontiers in Public Health*, 2025. DOI: https://doi.org/10.3389/fpubh.2025.1558716.

63. XIAO, B. et al. Dynamic analysis of SARS-CoV-2 evolution based on different countries. *Gene*, v. 916, p. 148426, 2024. DOI: https://doi.org/10.1016/j.gene.2024.148426.

7 APÊNDICE

7.1. Outras Atividades Desenvolvidas no Período do Doutorado

Durante o doutorado em Ciências Biológicas (Imunologia e Doenças Infecto-Parasitárias) pela Universidade Federal de Juiz de Fora (UFJF), atuei como analista no Laboratório de Diagnóstico Molecular de Doenças Infecciosas do ICB/UFJF, centro colaborador da Rede Estadual de Laboratórios Públicos de Minas Gerais (RESLP/CES-MG). Também participei como avaliadora científica em congressos nacionais e como membro de bancas examinadoras em trabalhos de conclusão de curso de graduação. Atuei ainda como avaliadora de projetos de iniciação científica (BIC/PIBIC) na Faculdade de Medicina de São José do Rio Preto-SP, além de integrar o comitê institucionais de avaliação de projetos e retorno às atividades de pesquisa durante a pandemia de COVID-19 na UFJF.

7.2 Ministração de Aulas

Entre 2021 e 2022, no âmbito do Programa de Incentivo ao Exercício do Ensino (PIEE/UFJF), lecionei Ciências e Biologia no Colégio de Aplicação João XXIII, atuando no Ensino Fundamental e Médio. Também ministrei diversas aulas e palestras relacionadas à virologia em congressos, e simpósios regionais, destacando-se: Novo Coronavírus e os desafios diagnósticos (2020), Novas variantes do SARS-CoV-2 e seus impactos (2021) e SARS-CoV-2 e a COVID longa (2024).

7.3 Participação em Congressos e Eventos

Participei ativamente de congressos, simpósios e seminários nacionais, apresentando trabalhos e palestras em diferentes áreas da virologia. Entre os principais: 4º Congresso de Microbiologia da LAMIC/UFJF (2025), II Semana de Ciências Farmacêuticas da UFJF (2024), 32º Congresso Brasileiro de Microbiologia (2023), além de congressos de virologia, imunização e simpósios temáticos entre 2020 e 2021. Obtive premiações por melhor pôster e melhor apresentação oral (IV Congresso da Liga Acadêmica de Microbiologia, 2025) e pelo melhor trabalho na área de Gestão em Saúde (II Semana de Ciências Farmacêuticas da UFJF, 2024).

7.4 Cursos e Capacitações

Participei de cursos e encontros técnicos-científicos em virologia, imunologia, diagnóstico molecular e imunizações, incluindo formações da Sociedade Brasileira de Imunização (SBIm) sobre vacinas contra COVID-19, além de workshops sobre sequenciamento de nova geração e biotecnologia viral.

7.5 Coorientações

Atuei como coorientadora em dois mestrados e quatro iniciações científicas. No mestrado, coorientei os trabalhos: Aspectos epidemiológicos e fingerprinting da microbiota respiratória em pacientes com COVID-19 e SRAG (2023) e Abordagem nutricional e microbiota intestinal na obesidade em adolescentes (2024). Na iniciação científica, coorientei estudos sobre epidemiologia e biologia molecular da COVID-19 (2024), perfil clínico-epidemiológico e status vacinal em SRAG/COVID-19 (2023), ocorrência de rinovírus humano (2022) e ocorrência de vírus sincicial respiratório (2022).

7.6 Participação em Projetos

Durante o doutorado, participei ativamente de projetos de pesquisa, desenvolvimento tecnológico, ensino e extensão, com destaque para: Epidemiologia e biologia molecular da COVID-19 na Zona da Mata Mineira (2020–atual), Vigilância sentinela de vírus respiratórios em crianças e adultos (2025–atual), Bioprospecção de cianometabólitos antivirais (2025–atual), Saúde Única na investigação de doenças infecciosas (2025–atual), Vigilância metagenômica viral em pacientes com doença febril aguda (2025–atual), Validação de aplicativo para vigilância participativa da dengue em Juiz de Fora (2025–atual) e o projeto de extensão 'Todos contra o novo coronavírus' (2021– 2024).

8 ANEXOS

8.1. ANEXO 1: PARECER CONSUBSTANCIADO DO CEP Nº 31527720.3.0000.5147

UFJF - UNIVERSIDADE FEDERAL DE JUIZ DE FORA - MG

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Aspectos epidemiológicos e moleculares da COVID-19 na Zona da Mata Mineira, construção de vetor recombinante com potencial para vacina oral e interferência do SARS-CoV-2 na estrutura da microbiota do trato respiratório superior, com implicações na evolução da doença.

Pesquisador: Vanessa Cordeiro Dias

Área Temática:

Versão: 4

CAAE: 31527720.3.0000.5147

Instituição Proponente: Departamento de Parasitologia, Microbiologia e Imunologia/UFJF

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.743.435

Apresentação do Projeto:

Trata-se da segunda versão de emenda acrescida ao projeto intitulado "Aspectos epidemiológicos e moleculares da COVID-19 na Zona da Mata Mineira, construção de vetor recombinante com potencial para vacina oral e interferência do SARS-CoV-2 na estrutura da microbiota do trato respiratório superior, com implicações na evolução da doença". A justificativa da emenda foi redigida como se segue: "Solicitamos essa emenda por necessidade de alargamento de prazos anteriormente previstos, seja por demora no recebimento de insumos e reagentes por vezes importados, seja por dificuldade de acesso ao laboratório de pesquisa em decorrência do cenário epidemiológico local motivado pela COVID-19. Ademais, um projeto de pesquisa dessa grandiosidade, com tantos experimentos complexos, demanda de mais recurso humano, que poderá ser acrescido de alunos de pós-graduação e graduação, logo que possível, conforme deliberação da UFJF. A inclusão o espécime clínico "líquor" se faz necessária em virtude do ineditismo do achado do SARS-CoV-2 neste tipo de amostra. Esse material já faz parte do nosso biorepositório, como requisito para elucidar o diagnóstico destes participantes e agora sustentará o mapeamento das variantes circulantes em nossa região e o entendimento da epidemiologia destas infecções em sistema nervoso central".

Endereço: JOSE LOURENCO KELMER S/N
Bairro: SAO PEDRO **CEP:** 36.036-900
UF: MG **Município:** JUIZ DE FORA
Telefone: (32)2102-3788 **Fax:** (32)1102-3788 **E-mail:** cep.propesq@ufjf.edu.br

Objetivo da Pesquisa:

São mantidos inalterados os objetivos enunciados em projeto já aprovado.

"Objetivo Primário:

Avaliar informações epidemiológicas, através de observação em prontuários eletrônicos (sexo, idade, grupo de risco, dentre outras) e clínicas (febre,

coriza, falta de ar) de indivíduos cujos espécimes clínicos serão recebidos no Laboratório de Fisiologia e Genética Molecular Microbiana, atualmente

credenciado pela vigilância sanitária como Laboratório de Diagnóstico da COVID-19 em Juiz de Fora, com resultado positivo ou negativo para SARS

-CoV-2. A partir destes espécimes clínicos, efetuar diagnóstico diferencial dos vírus influenza, vírus sincicial respiratório e rinovírus humano através

de PCR em tempo real, obter o RNA viral e o DNA metagenômico representativo, e assim realizar o sequenciamento do genoma viral e

metataxonômico para comparação da estrutura da microbiota do trato respiratório superior dos indivíduos com diagnostico positivo de COVID-19 e

daqueles portadores de síndrome respiratória por outras etiologias (negativos no teste molecular para detecção de SARS-CoV-2.

Objetivo Secundário:

a) Obter o genoma completo de linhagens virais representativas após sequenciamento do RNA viral, a partir de alíquotas de amostra de secreção de

trato respiratório superior (swab de naso/orofaringe; lavado de nasofaringe; aspirado de nasofaringe; aspirado broncoalveolar); e/ou amostras de

líquor de participantes com suspeita clinica e indicação de diagnóstico por PCR em tempo real, em Laboratório da UFJF credenciado pela Vigilância

Sanitária de Juiz de Fora da UFJF para avaliação da diversidade genética do SARS-CoV-2 em amostras positivas;

b) Obter o DNA metagenômico dos espécimes clínicos e determinar a carga de Streptococcuspneumoniae, Haemophilus

influenza,Micoplasmapneumoniae, Moraxellacatarralis, Enterobacteriaceae, Staphylococcussp.; realizar o sequenciamento a partir do DNA

metagenômico obtido, para correlação da quantificação bacteriana com os dados metataxonômicos e condição clínica dos indivíduos com

diagnostico positivo de COVID-19 ou síndrome respiratória por outras etiologias.

c) Identificar nos genomas obtidos, baseado na literatura, genes codificadores para proteínas de SARS-CoV-2 com potencial vacinal, realizar
amplificação e clonagem dos genes candidatos em vetores de expressão heteróloga e avaliar a expressão e estabilidade dos vetores construídos a
partir linhagens de Bactérias do Ácido Láctico (BAL) para uso como vacinais orais contra SARS-COV-2."

Avaliação dos Riscos e Benefícios:

A redação de riscos e benefícios é a mesma de projeto anteriormente aprovado."A pesquisa proposta é de risco mínimo, onde prevê-se a avaliação de dados clínicos (A consulta de dados se dará a partir de informações contidas
no formulário impresso disponível no site: www.saude.gov.br/influenza), sem divulgação da identidade dos participantes. Para minimizar os riscos,
todos os prontuários serão codificados, garantindo o anonimato dos participantes. Esta pesquisa será baseada na utilização de espécimes clínicos
que já farão parte da conduta médica, ou seja, o espécime já chegará ao laboratório coletado pela equipe de assistência médica (independente da
nossa equipe de pesquisa), como procedimento necessário ao diagnóstico da COVID-19 e assistência à saúde destes indivíduos. Após a realização
do diagnóstico, uma alíquota excedente do espécime, antes do descarte, será utilizada para a pesquisa proposta, o que não ocasiona, nesse caso,
risco direto ao participante.

Benefícios:

Construção de um banco de dados acerca da epidemiologia da infecção por COVID-19 e outros vírus respiratórios em Juiz de Fora - Minas Gerais e
região, bem como de tipos virais circulantes e de microbiota das vias aéreas superiores desses participantes. Assim, essas informações poderão
contribuir para implementação de estratégias de diagnóstico rápido e assertivo. Diante dessa emergência em saúde pública mundial, outro benefício
desta pesquisa consiste na identificação de possíveis genes codificadores para proteínas de SARS-CoV-2 com potencial vacinal, construídos a partir
de linhagens de Bactérias do Ácido Láctico (BAL), para uso como vacinais orais contra SARS-CoV-2."

Comentários e Considerações sobre a Pesquisa:

A pesquisadora justifica a necessidade da emenda, invocando a inevitabilidade de se estenderem os prazos pela demora no recebimento de insumos e reagentes e por limitações impostas pelo atual cenário epidemiológico.

Considerações sobre os Termos de apresentação obrigatória:

Sem considerações a acrescentar.

Conclusões ou Pendências e Lista de Inadequações:

Diante do exposto, a emenda ao projeto está aprovada, pois está de acordo com os princípios éticos norteadores da ética em pesquisa estabelecido na Res. 466/12 CNS e com a Norma Operacional Nº 001/2013 CNS. Data prevista para o término da pesquisa: dezembro de 2024.

Considerações Finais a critério do CEP:

Diante do exposto, o Comitê de Ética em Pesquisa CEP/UFJF, de acordo com as atribuições definidas na Res. CNS 466/12 e com a Norma Operacional Nº001/2013 CNS, manifesta-se pela APROVAÇÃO a emenda amostra. Esse material já faz parte do nosso biorepositório, como requisito para elucidar o diagnóstico destes participantes e agora sustentará o

mapeamento das variantes circulantes em nossa região e o entendimento da epidemiologia destas infecções em sistema nervoso central.". Vale lembrar ao pesquisador responsável pelo projeto, o compromisso de envio ao CEP de relatórios parciais e/ou total de sua pesquisa informando o andamento da mesma, comunicando também eventos adversos e eventuais modificações no protocolo.

amostra. Esse material já faz parte do nosso biorepositório, como requisito para elucidar o diagnóstico destes participantes e agora sustentará o

mapeamento das variantes circulantes em nossa região e o entendimento da epidemiologia destas infecções em sistema nervoso central.". Vale lembrar ao pesquisador responsável pelo projeto, o compromisso de envio ao CEP de relatórios parciais e/ou total de sua pesquisa informando o andamento da mesma, comunicando também eventos adversos e eventuais modificações no protocolo.

O presente projeto, seguiu nesta data para análise da CONEP e só tem o seu início autorizado após a aprovação pela mesma.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_1729645_E1.pdf	19/05/2021 16:19:53		Aceito
Outros	Carta_resposta_2.docx	19/05/2021 16:17:12	Vanessa Cordeiro Dias	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Emenda_2.docx	19/05/2021 16:10:55	Vanessa Cordeiro Dias	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Dispensa_TCLE_2.doc	19/05/2021 16:09:30	Vanessa Cordeiro Dias	Aceito
Outros	img051.pdf	25/05/2020 21:12:11	Vanessa Cordeiro Dias	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Biorepositorio.pdf	06/05/2020 21:33:53	Vanessa Cordeiro Dias	Aceito
Folha de Rosto	Folha_de_rosto.pdf	06/05/2020 21:32:32	Vanessa Cordeiro Dias	Aceito
Declaração do Patrocinador	Patrocinador.doc	26/04/2020 23:21:09	Vanessa Cordeiro Dias	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Sim

JUIZ DE FORA, 28 de Maio de 2021

Assinado por:
Jubel Barreto
(Coordenador(a))

8.2 ANEXO 2: PARECER CONSUBSTANCIADO DO CEP Nº 70133723.3.0000.5147

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do resistoma clínico em amostras do trato respiratório de indivíduos com resultado positivo ou negativo para SARS-CoV-2, virus Influenza e Virus Sincicial Respiratório (VSR)

Pesquisador: Vânia Lúcia Silva

Área Temática:

Versão: 1

CAAE: 70133723.3.0000.5147

Instituição Proponente: Departamento de Parasitologia, Microbiologia e Imunologia/UFJF

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.167.235

Apresentação do Projeto:

As informações elencadas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas do arquivo Informações Básicas da Pesquisa (PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2150912.pdf 01/06/2023 11:00:12)

Desenho:

"Estudo transversal, prospectivo, onde será avaliado o resistoma bacteriano de amostras de trato respiratório de indivíduos com suspeita de SARSCoV-2, Influenza e/ou Vírus Sincicial Respiratório, em municípios (microrregiões de Barbacena, Além Paraíba, Leopoldina e Cataguases), hospitalizados ou não, maiores de 18 anos, cujos espécimes clínicos (swabs de nasofaringe e orofaringe; lavado de nasofaringe; aspirado de nasofaringe; aspirado broncoalveolar, lavado brônquico) forem enviados ao CEMIC – Centro de Estudos em Microbiologia -UFJF, para diagnostico molecular das viroses. O CEMIC foi credenciado junto à Secretaria Estadual de Saúde (SES-MG) e vistoriado pela Vigilância Sanitária em Juiz de Fora como Centro colaborador no diagnóstico molecular de doenças respiratórias e arboviroses."

Introdução:

"Patógenos emergentes e reemergentes são desafios globais para a saúde pública (Gao, 2018). As infecções respiratórias sempre foram bastante frequentes nos serviços de saúde no Brasil. Nos Estados Unidos, está entre as cinco mais frequentes em pessoas acima de 65 anos, e é ainda

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considerada a principal causa de morte nos países em desenvolvimento. Além da mortalidade, o impacto das infecções do trato respiratório traduz se no prolongamento da hospitalização e aumento dos custos (Silva et al., 2011). Um parâmetro bastante utilizado para avaliar a gravidade de uma infecção respiratória viral é a internação em UTI. Dentre os vírus que podem acometer o trato respiratório e podem causar infecção grave, devido sua maior frequência em pacientes que necessitam de internação em UTI, maior frequência de complicações e maiores taxas de mortalidade, destacam-se: vírus Influenza (Wiemken, 2013; Sundakar, 2011), rinovírus humano (Daubin, 2006; Kraft, 2012) e o vírus sincicial respiratório (VSR) (Wiemken, 2013; Schnell 2014). A esta lista é possível acrescentar o SARS-CoV-2, agente etiológico da COVID-19, doença que vitimou milhões de

pessoas em todo o mundo, sendo responsável pela maior pandemia da história da humanidade (Peeling et al., 2022).A patogênese das infecções respiratórias envolve uma complexa interação entre patógeno, hospedeiro, e variáveis epidemiológicas que facilitam esta dinâmica. Vários mecanismos contribuem para a ocorrência destas infecções, porém o papel de cada um destes fatores ainda permanece controverso, podendo variar de acordo com a população envolvida e o agente etiológico (Brasil, 2013).Apesar de as viroses respiratórias poderem evoluir de maneira grave com altas taxas de mortalidade e morbidade, particularmente em idades extremas, a sua fisiopatologia ainda não foi completamente elucidada por envolver interações com outros microrganismos e imunológicas complexas, muitas vezes negligenciadas. O uso indiscriminado de drogas antimicrobianas resultou na emergência de patógenos bacterianos clássicos e multirresistentes, que já não respondem às terapias tradicionais. De acordo com a OMS, a resistência aos antimicrobianos é um problema global, urgente e complexo, que configura um dos maiores desafios da saúde

pública na atualidade (WHO, 2020b). O surgimento de microrganismos multirresistentes resulta em infecções que respondem a poucos ou nenhum dos agentes antimicrobianos disponíveis no mercado, o que leva alguns autores a acreditar na eminência de uma segunda era pré-antibiótica (APPELBAUM, 2012). Ao longo dos últimos anos, a incidência de infecções por microrganismos resistentes tem aumentado e, atualmente, estima-se que 700.000 pessoas morram a cada ano devido à resistência antimicrobiana em todo o mundo. Se o problema não for abordado, esse número pode chegar a 10 milhões em 2050, ao custo de 100 trilhões de dólares para a economia mundial (O'NEILL, 2016). Apesar da resistência aos antimicrobianos ser um fenômeno que ocorre naturalmente, como resultado da seleção natural, atividades antropogênicas tem contribuído para o aumento da frequência com que ela ocorre

(WILLERS et al., 2017). A prescrição excessiva ou inadequada de antimicrobianos na medicina humana e o seu uso incorreto por pacientes favorecem a pressão seletiva em patógenos e microrganismos comensais e, consequentemente, a propagação de linhagens resistentes (FAIR; TOR, 2014; ROCA et al., 2015)..O desenvolvimento de técnicas independentes de cultivo revelou uma nova abordagem para o conhecimento dos determinantes genéticos relacionados à resistência aos antimicrobianos, e revelou o impacto que tem produzido o surgimento deste fenômeno (GRICE; SEGRE, 2012). Foi proposto então o termo resistoma, definido pelo conjunto dos genes de resistência a antimicrobianos, principalmente de bactérias, relacionado à porção do pangenoma (totalidade genes presentes em todos os genomas de todos os procariotas na biosfera) em um determinado ecossistema. Dentro do resistoma, os genes de resistência aos antimicrobianos de importância clínica são definidos como resistoma clínico, que pode ser relacionado à medicina humana ou animal (FORSLUND et al., 2013; VERSLUIS et al., 2015).Em estudos epidemiológicos, é de extrema importância a avaliação do resistoma clinico, considerando-se que as bactérias que habitam normalmente a microbiota de seres humanos podem carrear ou albergar grande diversidade de marcadores genéticos. Este processo é agravado pelo tratamento oral com drogas antimicrobianas de amplo espectro, direta ou indiretamente, que tem efeito também nas bactérias da microbiota residente, enriquecendo o reservatório de genes de resistência disponível para os patógenos (WILLMANN et al., 2015). Por outro lado, em uma abordagem contemporânea, a resistência bacteriana,fúngica e viral aos antimicrobianos tem ganhado destaque nas discussões mais amplas que envolvem saúde pública. É relatado que cerca de 700 mil pessoas/ano morrem de infecções por microrganismos resistentes a drogas com previsão de 10 milhões de mortes/ano até 2050. A partir de 2020, a pandemia passa a contribuir na questão de forma ainda não mensurada: 70-80% dos pacientes com COVID-19 receberam antimicrobianos empiricamente [27]. O custo da resistência aos antimicrobianos para a economia mundial é significativo. Além de morte e invalidez, doenças por
microrganismos resistentes resultam em internações prolongadas, necessidade de medicamentos extras e em desafios financeiros que requerem ações multissetoriais (Rawson et al., 2020). A OMS declarou a resistência aos antimicrobianos como uma das principais ameaças à saúde pública no sec. XXI. Há um consenso global sobre o problema e sua importância. Com o surgimento da COVID-19, e recentemente o aumento das síndromes respiratórias, o aumento de uso empírico de antimicrobianos tem retardado a adoção de medidas de contenção. Durante a pandemia, entre os pacientes com COVID-19 que tem recebido antimicrobianos, aqueles que têm coinfecção na internação não superam 5% e intercorrências

infecciosas cheguem a 15% na hospitalização. O entendimento dos impactos da pandemia no fenômeno, sobretudo localmente poderá subsidiar políticas de enfrentamento e uso racional de drogas para mitigação das possíveis consequências da resistência aos antimicrobianos em curto e médio prazo no pós-pandemia (Miranda et al., 2020)."

Hipótese:

"O conhecimento dos marcadores de resistência aos antimicrobianos representativo do resistoma clinico do trato respiratório podem predizer aspectos da utilização indevida ou indiscriminada de antimicrobianos para o tratamento de doenças respiratórias, com implicações no curso e no agravo da doença."

Metodologia Proposta:

"Recepção das amostras dos indivíduos com suspeita de viroses respiratórias: Após uma parceria da UFJF com a Secretaria de Estado de Saúde de MG - SES/MG, o CEMIC - Centro de Estudos em Microbiologia do ICB, se responsabilizou, dentro do programa de Vigilância Laboratorial, pela realização de diagnóstico molecular de viroses respiratórias (síndromes gripais, Influenza, COVID-19, etc) a partir de demanda de municípios (microrregiões de Barbacena, Além Paraíba, Leopoldina e Cataguases), que nos enviarão os espécimes clínicos para a realização dos procedimentos. O CEMIC já possui Alvará Sanitário para o diagnóstico molecular de doenças infecto-contagiosas desde o enfrentamento da COVID-19 na UFJF, e está preparado para receber os tubos de coleta com os swabs de nasofaringe e orofaringe; lavado de nasofaringe; aspirado de nasofaringe; aspirado broncoalveolar; lavado brônquico de pacientes do sistema público de saúde (SUS). A coleta deverá ser realizada por profissionais de saúde treinados, nas respectivas unidades de saúde e/ou hospitais, e com a utilização de todos os equipamentos de proteção individual (EPI) para contenção de vírus transmitidos via respiratória (gotículas e aerossol). Essas coletas independem da nossa equipe de pesquisa, ou seja, nossa equipe não terá participação na coleta dos espécimes clínicos. As amostras serão recebidas das microrregiões de saúde de Barbacena, Além

Paraíba, Leopoldina e Cataguases, conforme acordado em contrato com a SES/MG. Após a coleta nas cidades, os tubos serão transportados ao CEMIC, na UFJF, em recipiente específico, lacrados e refrigerados a 4ºC, no menor espaço de tempo possível. Amostras de 300 uL dos espécimes clínicos serão aliquotadas e armazenadas a -80º C para a realização apenas da parte experimental prevista neste projeto, e o restante das amostras seguirá para o diagnóstico molecular das viroses respiratórias. Extração de DNA metagenômico bacterianoO DNA metagenômico bacteriano dos espécimes clínicos do trato respiratório será extraído a partir de alíquotas de 300 uL, previamente

armazenadas sob congelamento. Será utilizado o mini kit QIAamp™ DNA Blood (Qiagen, Hilden, Alemanha), de acordo com as instruções do fabricante. A concentração e a pureza do DNA serão determinadas por espectrofotometria usando o aparelho Nano Drop 2000c, utilizando-se a DO 260 nm e a relação de leituras a 260 nm e a 280 nm para estimar a pureza do DNA, estabelecendo-se o valor de 1,75 como referência mínima. A integridade do DNA será avaliada por eletroforese em gel de agarose a 0,8% em tampão TBE (Tris-HCl-Borato- EDTA). Os extratos de DNA serão aliquotados e armazenados em freezer a -80°C, até o momento da utilização. Pesquisa de genes de resistência aos antimicrobianos do resistoma clinico Serão pesquisados por PCR convencional os marcadores de resistência representativos do resistoma clínico: blaCTX-M, blaKPC, blaSHV, blaTEM, blaOXA23, blaOXA51, blaOXA24, blaOXA143,blaOXA58, blaOXA10, blaOXA2, blaZ, cfxA/cfxA2, ampC, blaVIM, blaNDM-1, blaSPM-1, cfiA, tet(A), tet(B), tet(E), tet(K), tet(L), tet(M), tet(O),tet(Q),mrsA, mecA, mef, ereA, ereB, mphA, ermA, ermB, ermC, qnrB, qnrS, sul1, sul2, sul3, nim1, nim2, nim3, nim4, aacA-aphD, vatA, vatB, vatC, vga,
vgb, linA, mrsB, cepA, mexB, mexD, mexF, mexY. Serão utilizados pares de primers específicos e condições de amplificação já descritas na literatura (SARMIENTO et al., 2019) em termociclador automatizado, programado nas seguintes condições: desnaturação inicial a 94°C por 5 minutos; 30 ciclos de desnaturação a 94°C por 1 minuto, anelamento a 50°C por 1 minuto e extensão a 72°C por 2,5 minutos; extensão final a 72°C por 10 minutos. Os amplicons serão separados em gel de agarose 2% utilizando tampão TAE (Tris-Acetato-EDTA), por 2 horas a 100 volts. Posteriormente, o gel será corado com brometo de etídio e visualizado em transiluminador de luz ultravioleta. Será utilizado o padrão de peso
molecular 100pb DNA."

Critério de Inclusão:

"Espécimes clínicos de trato respiratório de indivíduos sintomáticos (tosse, falta de ar, coriza, febre, síndrome respiratória aguda), hospitalizados ou não, maiores de 18 anos, cujos espécimes clínicos forem enviados para o diagnóstico molecular de viroses no CEMIC/UFJF."

Critério de Exclusão:

"Espécimes clínicos que não estiverem adequados no momento da recepção das amostras no laboratório (tubos de coleta sem o swab, tubos não vedados de maneira apropriada,espécimes clínicos sem identificação)."

Serão 2.500 amostras, sendo 1250 de Espécimes clínicos - teste negativo e 1250 de Espécimes clínicos - teste positivo, as intervenções serão Extração de DNA bacteriano metagenômico e avaliação dos genes de resistência aos antimicrobianos nos espécimes clínicos.

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Objetivo da Pesquisa:

Objetivo Primário:

"Avaliar comparativamente o resistoma dos indivíduos que passaram por terapia antimicrobiana empírica (comunitária ou hospitalar), a partir de amostras do trato respiratório de indivíduos cujos espécimes clínicos serão recebidos no CEMIC – Centro de Estudos em Microbiologia, atualmente credenciado pela Vigilância Sanitária como Laboratório de Diagnóstico Molecular de Doenças Infecto-contagiosas, com resultado positivo ou negativo para SARS-CoV-2, vírus Influenza ou Virus sincicial respiratório (VSR)."

Objetivo Secundário:

• Extrair DNA bacteriano metagenômico a partir dos espécimes clínicos do trato respiratório obtidos para o diagnóstico molecular;

• Avaliar a presença de marcadores de resistência bacterianos a drogas antimicrobianas representativas do resistoma clínico nos DNAs metagenômicos obtidos;

• Correlacionar a distribuição dos marcadores de resistência a drogas antimicrobianas entre os indivíduos com resultado positivo para SARS-CoV-2, Influenza ou VSR com aqueles indivíduos com resultado negativo para estas viroses respiratórias.

Avaliação dos Riscos e Benefícios:

Riscos:

"Esta pesquisa será baseada na utilização de espécimes clínicos do trato respiratório superior e inferior, que já farão parte da conduta médica, ou seja, o espécime já chegará ao laboratório coletado pela equipe de assistência médica (independente da nossa equipe de pesquisa), como procedimento necessário ao diagnóstico molecular, que será realizado em nosso laboratório. Uma parte do espécime clínico será alíquota da para ser utilizada na pesquisa proposta. Dessa forma, a pesquisa caracteriza-se como sendo de risco mínimo, que poderia envolver o risco de identificação dos participantes. Para mitigar os riscos, os espécimes clínicos serão codificados, no recebimento das coletas, para garantir o anonimato dos participantes."

Benefícios:

"Construção de um banco de dados acerca da epidemiologia dos marcadores de resistência bacteriana aos antimicrobianos representativos do resistoma clínico dos participantes com resultado positivo ou negativo para as viroses respiratórias. Assim, essas informações poderão contribuir para implementação de estratégias de voltadas para o gerenciamento do uso indiscriminado de antimicrobianos, além de suscitar políticas públicas visando a promoção de

saúde e saneamento."

Comentários e Considerações sobre a Pesquisa:

Vide 'Conclusões ou Pendências e Lista de Inadequações'

Considerações sobre os Termos de apresentação obrigatória:

O protocolo de pesquisa apresenta FOLHA DE ROSTO devidamente preenchida,com o título em português, identifica o patrocinador pela pesquisa, estando de acordo com as atribuições definidas na Norma Operacional CNS 001 de 2013 item 3.3 letra a; e 3.4.1 item 16.

Não apresenta o INSTRUMENTO DE COLETA DE DADOS, mas na metodologia detalha como será conduzida a pesquisa(análise das amostras de espécimes clínicos) de forma pertinente aos objetivos delineados e preserva os participantes da pesquisa.

O Pesquisador apresenta titulação e experiência compatível com o projeto de pesquisa, estando de acordo com as atribuições definidas no Manual Operacional para CEPs.

Apresenta DECLARAÇÃO infraestrutura do biorrepositorio e concordância com a realização da pesquisa de acordo com as atribuições definidas na Norma Operacional CNS 001 de 2013 item 3.3 letra h.

Apresenta Termo de sigilo devidamente redigido e assinado pelo pesquisador responsável.

Apresenta Contrato da SES-MG - Instrumento Legalpara repasse de recursos deliberado CIB-SUS/MG n. 3,681 de 16/12/2021. Total de recursos:R$1.180.869,42. Assinado pelo responsável pela UFJF em 23/03/2022.

Apresenta Declaração da coordenadora do Diagnóstico Molecular de Doenças infecciosas junto a Secretaria de Estado de Saúde de Minas Gerais – SES/MG, dentro do programa de Vigilância Laboratorial, e em parceria com a UFJF, aquiescente com a cessão de parte dos espécimes clínicos que serão enviados ao CEMIC para o diagnóstico de viroses respiratórias, para a pesquisa intitulada: Avaliação do resistoma clínico em amostras do trato respiratório de indivíduos com resultado positivo ou negativo para SARS-CoV-2, virus Influenza e Virus Sincicial Respiratório (VSR).

Apresenta Termo de Dispensa de TCLE, assim justiçado: "Este estudo será realizado após uma parceria da UFJF com a Secretaria de Estado de Saúde de MG - SES/MG, onde o CEMIC se responsabilizou pela realização de diagnóstico molecular de viroses respiratórias a partir de demanda de municípios (regionais de Barbacena e Leopoldina), que nos enviarão os espécimes clínicos para a realização dos procedimentos. Desta forma, os pesquisadores na UFJF não participarão da coleta das amostras e não terão como abordar, explicar e orientar os indivíduos

sobre o a utilização das amostras para esta pesquisa. Assim, solicitamos a dispensa de TCLE. A utilização de ferramentas eletrônicas, como SMS, mensagens e formulários eletrônicos tipo GOOGLE FORMS também não se mostra efetiva, considerando que parte da população atendida é de zona rural ou de cidades ainda com pouco desenvolvimento. Entendendo que a pesquisa proposta possui grande relevância em termos de saúde pública e que os pesquisadores se comprometem com o anonimato dos participantes e sigilo dos dados, associado ao fato de que todos os pesquisadores assinaram termo de sigilo e confidencialidade junto à SES/MG, solicita-se a dispensa de TCLE."

Como explicitado, a equipe de pesquisa não participará da coleta de espécimes clínicas (swabs de nasofaringe e orofaringe; lavado de nasofaringe; aspirado de nasofaringe; aspirado broncoalveolar, lavado brônquico). A coleta será realizada por profissionais de saúde treinados, nas respectivas unidades de saúde e/ou hospitais, e com a utilização de todos os equipamentos de proteção individual (EPI) para contenção de vírus transmitidos via respiratória (gotículas e aerossol). SENDO ASSIM, ESTES PROFISSIONAIS PODERÃO OBTER DOS DOADORES DE ESPÉCIMES CLÍNICAS O TCLE.

Recomendações:

Não se aplica.

Conclusões ou Pendências e Lista de Inadequações:

Pendências:

1) Apresentar cronograma para realização do estudo proposto e não para 'Extração de DNA metagenômico bacteriano do material fecal'.

2) Apresentar TCLE. Como explicitado, a equipe de pesquisa não participará da coleta de espécimes clínicas (swabs de nasofaringe e orofaringe; lavado de nasofaringe; aspirado de nasofaringe; aspirado broncoalveolar, lavado brônquico). A coleta será realizada por profissionais de saúde treinados, nas respectivas unidades de saúde e/ou hospitais, e com a utilização de todos os equipamentos de proteção individual (EPI) para contenção de vírus transmitidos via respiratória (gotículas e aerossol). Sendo assim, estes profissionais poderão obter dos doadores de espécimes clínicas o TCLE.

3) Como os profissionais de saúde que coletarão espécimes clínicas não pertencem a equipe de pesquisa, o pesquisador responsável deve apresentar a Declaração de aquiescência e compromisso dos mesmos.

Considerações Finais a critério do CEP:

Diante do exposto, o Comitê de Ética em Pesquisa CEP/UFJF, de acordo com as atribuições definidas na Res. CNS 466/12 e com a Norma Operacional Nº 001/2013 CNS, manifesta-se pela PENDÊNCIA do protocolo de pesquisa proposto, devendo o pesquisador providenciar as correções listadas, em um prazo de 30 (trinta) dias, para que possamos liberar o parecer.COM O INTUITO DE AGILIZAR O PROCESSO DE TRAMITAÇÃO DOS PROJETOS DE PESQUISA NO CEP, HÁ OBRIGATORIEDADE DE O(A) PESQUISADOR(A) :

1) DESCREVER CLARAMENTE NO CAMPO "OUTRAS INFORMAÇÕES, JUSTIFICATIVAS OU CONSIDERAÇÕES A CRITÉRIO DO PESQUISADOR", OS ITENS A e B:

A) CADA ALTERAÇÃO REALIZADA PARA CADA PENDÊNCIA APONTADA;

B) JUSTIFICATIVA PARA CADA PENDÊNCIA NÃO ATENDIDA, QUANDO FOR O CASO;

2) REDIGIR ESSAS MESMAS ALTERAÇÕES NOS DOCUMENTOS PERTINENTES CONSTANTES DO PROTOCOLO DO PROJETO DE PESQUISA;

3) DESTACAR EM AMARELO ESSAS ALTERAÇÕES (O DESTAQUE EM AMARELO SÓ NÃO É POSSÍVEL NA DIGITAÇÃO DE "INFORMAÇÔES BÁSICAS DO PROJETO" NO SISTEMA DA PLATAFORMA BRASIL).

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2150912.pdf	01/06/2023 11:00:12		Aceito
Outros	curriculopesquisadoresviroses.pdf	01/06/2023 10:59:43	Vânia Lúcia Silva	Aceito
Outros	declaracaocoordenadora.pdf	31/05/2023 12:59:17	Vânia Lúcia Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	DispensaTCLE.pdf	31/05/2023 12:57:22	Vânia Lúcia Silva	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	declaracaoinfraestruturabiorrepositorio.pdf	31/05/2023 12:52:56	Vânia Lúcia Silva	Aceito
Folha de Rosto	folhaDeRosto.pdf	31/05/2023 12:52:34	Vânia Lúcia Silva	Aceito
Outros	contratoSES.pdf	31/05/2023	Vânia Lúcia Silva	Aceito

Continuação do Parecer: 6.167.235

Outros	contratoSES.pdf	11:59:08	Vânia Lúcia Silva	Aceito
Outros	Termodesigilo.pdf	31/05/2023 11:58:13	Vânia Lúcia Silva	Aceito
Projeto Detalhado / Brochura Investigador	projetodetalhado.pdf	31/05/2023 11:57:21	Vânia Lúcia Silva	Aceito

Situação do Parecer:
Pendente

Necessita Apreciação da CONEP:
Não

JUIZ DE FORA, 06 de Julho de 2023

Assinado por:
Patrícia Aparecida Baumgratz de Paula
(Coordenador(a))

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