Received: 19 January 2021 | Revised: 10 March 2021 | Accepted: 26 March 2021

DOI: 10.1002/jmv.26975

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Low circulation of Influenza A and coinfection with SARS-CoV-2 among other respiratory viruses during the COVID-19 pandemic in a region of southern Brazil

Ana Karolina Antunes Eisen MSc | Juliana Schons Gularte PhD | Meriane Demoliner MSc | Vyctoria Malayhka de Abreu Goés Pereira BSc | Fágner Henrique Heldt MSc | Micheli Filippi BSc | Paula Rodrigues de Almeida PhD | Alana Witt Hansen PhD | Juliane Deise Fleck PhD | Fernando Rosado Spilki PhD

Laboratório de Microbiologia Molecular, Instituto de Ciências da Saúde, Universidade Feevale, Novo Hamburgo, Rio Grande do Sul, Brazil

Correspondence
Ana Karolina Antunes Eisen, Laboratório de Microbiologia Molecular, Instituto de Ciências da Saúde, Universidade Feevale, ERS-239, 2755. CEP: 93525-075 Novo Hamburgo, Rio Grande do Sul, Brazil.
Email: akaeisen@gmail.com

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 302668/2018-7; Financiadora de Estudos e Projetos, Grant/Award Number: 01.20.0029.00

Abstract

With the arrival of coronavirus disease 2019 (COVID-19) in Brazil in February 2020, several preventive measures were taken by the population aiming to avoid severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection including the use of masks, social distancing, and frequent hand washing then, these measures may have contributed to preventing infection also by other respiratory viruses. Our goal was to determine the frequencies of Influenza A and B viruses (FLUAV/FLUBV), human mastadenovirus C (HAdV-C), Enterovirus 68 (EV-68), and rhinovirus (RV) besides SARS-CoV-2 among hospitalized patients suspect of COVID-19 with cases of acute respiratory disease syndrome (ARDS) in the period of March to December 2020 and to detect possible coinfections among them. Nucleic acid detection was performed using reverse-transcription quantitative polymerase chain reaction (RT-qPCR) in respiratory samples using naso-oropharyngeal swabs and bronchoalveolar lavage. A total of 418 samples of the 987 analyzed (42.3%) were positive for SARS-CoV-2, 16 (1.62%) samples were positive for FLUAV, no sample was positive for FLUBV or EV-68, 67 (6.78%) samples were positive for HAdV-C, 55 samples were positive for RV 1/2 (26.3%) and 37 for RV 2/2 (13.6%). Coinfections were also detected, including a triple coinfection with SARS-CoV-2, FLUAV, and HAdV-C. In the present work, a very low frequency of FLUV was reported among hospitalized patients with ARDS compared to the past years, probably due to preventive measures taken to avoid COVID-19 and the high influenza vaccination coverage in the region in which this study was performed.

KEYWORDS
ARDS, coinfection, COVID-19, EV-68, HAdV-C, influenza, rhinovirus

1 | INTRODUCTION

By the end of 2019, scientists announced the circulation of a new type of coronavirus in China, which was later officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belonging to the *Severe acute respiratory syndrome-related coronavirus* species together with the first SARS-CoV.[1] Coronavirus disease 2019 (COVID-19), the disease caused by it, was linked to a seafood market in the city of Wuhan, Hubei province, and since then, the disease quickly spread around the world causing 87.589.206 confirmed cases by January 9th and 1.906.606 deaths.[2-4] COVID-19 was rapidly declared a pandemic, in about 3 months since its beginning. Regarding being a burden for public health, the present pandemic is only comparable to the Spanish Flu back in 1918, caused by *Influenza A virus* (FLUAV) H1N1.[5]

Respiratory pathogens like viruses, bacteria, and fungi are commonly found infecting the same host simultaneously, in fact, coinfection with several different respiratory viruses is a common finding as well as viral-bacterial coinfection, mainly taking into account that seasonality overlaps in some of them.[6-8] Among respiratory viruses, FLUAV and *Influenza B virus* (FLUBV) species are prevalent, as well as *Rhinovirus* (RV) of *Enterovirus* genus and *Human mastadenovirus* C (HAdV-C).[8-12]

The advent of COVID-19 forced the adoption of several non-pharmacological preventive measures aiming to avoid SARS-CoV-2 infection like wearing masks, social distancing, and frequent hand washing. Furthermore, as an attempt to contain COVID-19 and maintain the economy, the state government created a system of "controlled distancing" which has been updated weekly through a system of color-based risk flags that represent a low risk (yellow), medium risk (orange), high risk (red) and very high risk (black). Depending on the flag's color, the measures required for commerce and services establishments are more restricted and some activities may be prohibited.[13,14] These measures may have contributed to preventing infection by other respiratory viruses besides SARS-CoV-2[15,16] and their circulation may have been reduced along their seasonal months in 2020.

Therefore, the goal of this study was to evaluate the frequency of other viral agents of respiratory illness, including FLUAV and FLUBV, RV, *Enterovirus* D68 (EV-68), and HAdV-C in samples collected from hospitalized patients suspected of acute respiratory distress syndrome (ARDS) by SARS-CoV-2 as well as to detect possible viral coinfections among them.

2 | MATERIALS AND METHODS

2.1 | Samples

Respiratory samples from 40 different municipalities of Rio Grande do Sul, the southernmost state of Brazil (Figure S1), were received for COVID-19 molecular diagnosis in Laboratório de Microbiologia Molecular of Universidade Feevale, Novo Hamburgo, RS, Brazil. Samples in this study were chosen randomly among clinical samples from hospitalized patients presenting typical cases of ARDS and suspected of having COVID-19. For this study, 987 cases were selected along a period comprising months of March to December of 2020. This corresponds to the previous phase of the first peak of COVID-19 cases in the region (which occurred in the end of July), to the beginning of the second wave that started in October 2020. Respiratory samples were naso-oropharyngeal swabs or bronchoalveolar lavage, collected by local healthcare professionals in sterile saline solution following standard guidelines, and transported to the laboratory under refrigeration until 24 h after sampling. This study was approved by the University's Ethical Review Board (protocol number: 33202820.7.1001.5348), following Brazilian regulations and international ethical standards.

2.2 | Nucleic acid extraction

Samples were stored at 4°C until the nucleic acid extraction was performed before completing 24 h. Extractions were made with a commercial kit MagMAX™ CORE Nucleic Acid Purification Kit (Applied biosystems™) using the automated equipment KingFisher™ Duo Prime (Thermo Fisher Scientific™). Saline was also extracted and used as a negative control of the extraction (CE) in each round to ensure the absence of contamination during this process. Nucleic acids were then stored at −80°C until further analysis.

2.3 | Nucleic acids amplification by reverse-transciption quantitative polymerase chain reaction (RT-qPCR)

FLUAV, FLUBV, types of FLUAV H3, SARS-CoV-2, HAdV-C, RV, and EV-D68 were aimed for detection by nucleic acid amplification. RT-qPCR reactions were made in a 25 µl total volume for FLUVs detection, comprising 12.5 µl of 2× RT-PCR buffer and 1 µl of 25X Enzyme mix of AgPath-ID™ One-Step RT-PCR Reagents (Thermo Fisher Scientific™), 0.5 µl of each primer and probe, 5 µl of nuclease free-water (NFW) and 5 µl of sample. SARS-CoV-2 detection reactions were made in a 20 µl total volume, comprising 10 and 0.8 µl of the same buffer and enzyme mix respectively, as well as 0.8 µl of each primer, 0.4 µl of the probe, 2.2 µl of NFW, and 5 µl of the sample. Amplification cycle was the same for FLUV and SARS-CoV-2 detection, beginning at 50°C for 15 min for reverse transcription, followed by denaturation at 95°C for 10 min and by 40 cycles of 95°C for 15 s and 60°C for 45 s. For HAdV-C detection, qPCR reactions were made in a 20 µl final volume, with 10 µl of Taqman® Fast Advanced Master Mix (Thermo Fisher Scientific™), 2 µl of NFW, 1 µl of each primer and probe, and 5 µl of the sample. Amplification cycle was according to Wolf et al.[17]

For RVs and EV-68 detection the primer-probe kit of TaqMan® Respiratory Tract Microbiota Profiling Experiments (Applied Biosystems™) was used in a total volume reaction of 15 µl, with 7.5 µl

of 2X RT-PCR Buffer, 0.6 µl of 25X Enzyme mix of AgPath-ID™ One-Step RT-PCR Reagents (Thermo Fisher Scientific™), 0.9 µl of NFW and 1 µl of primer-probe in each independent reaction, the human rhinovirus 1/2, human rhinovirus 2/2 and human enterovirus D68.

In all analyses, a no template control (NTC) was used as a negative control to ensure absence of contamination during the RT-qPCR process and positive controls were also used in all plaques to ensure the effectiveness of all reactions. Positive controls used were a FLUAV swine H1N1 pandemic virus isolated in MDCK cells (FLUAV), H3N2 virus isolated in MDCK cells (H3 types), a human FLUBV isolated in MDCK cells (FLUBV), HAdV-5 isolated in A549 cells (HAdV-C), and a poliovirus 2 sample (RV).

Descriptions of primers and probes with their references can be seen in Table 1, except for RVs and EV-68 which were not described in the kit of TaqMan® Respiratory Tract Microbiota Profiling Experiments.

3 | RESULTS

A total of 987 clinical samples from ARDS hospitalized patients were analyzed from March to December of 2020. Most samples were nasoorophary ngeal swabs (91.3%). Among the patients, 51.8% were male and 48.2% female from 41 different municipalities, although 46.2% of samples included in this study were from the same city, Novo Hamburgo. The patient included with the highest age was 99 years old and the lowest was 2 months old, most (59.4%) patients were elderly (>60 years old), 30.6% were adults (36–59 years old), 9.9% were young adults (19–35 years old) and 2.1% were children or teenagers (<18 years old), age and gender distribution are shown in Figure 1.

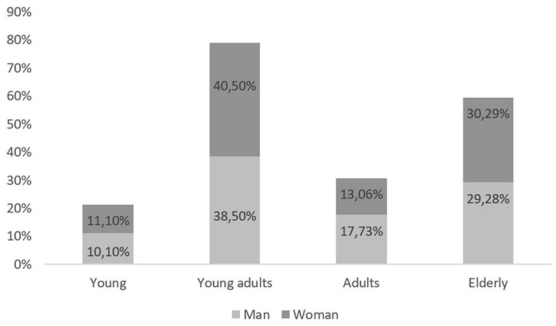

FIGURE 1 Age and gender distribution of 987 patients included in the study. Of the total, 51.8% were male and 48.2% were female, distributed further into young (≤18 years old), young adults (19–35), adults (36–59), and elderly (≥60)

TABLE 1 Descriptions of primers and probes used in SARS-CoV-2, FLUV, and HAdV-C detection

Oligonucleotides	Sequence 5'→ 3'	Concentration, µM	References
InfA primer F	GACCRATCCTGTCACCTCTGAC	40	CDC[18]
InfA primer R	AGGGCATTYTGGACAAAKCGTCTA	40	
InfA sonda	TGCAGTCCTCGCTCACTGGGCACG	10	
InfB primer F	TCCTCAAYTCACTCTTCGAGCG	40	Selvaraju and Selvarangan[19]
InfB primer R	CGGTGCTCTTGACCAAATTGG	40	
InfB sonda	CCAATTCGAGCAGCTGAAACTGCGGTG	10	
pdmH1primer F	GTGCTATAAACACCAGCCTYCCA	40	Rönkkö et al.[20]
pdmH1 primer R	CGGGATATTCCTTAATCCTGTRGC	40	
pdmH1sonda	CAGAATATACATCCRGTCACAATTGGARAA	10	
pdmInfA primer F	GCACGGTCAGCACTTATYCTRAG	40	CDC[18]
pdmInfA primer R	GTGRGCTGGGTTTTCATTTGGTC	40	
pdmInfA sonda	CYACTGCAAGCCCATACACACAAGCAGGCA	10	
H3 primer F	AAGCATTCCYAATGACAAACC	40	Tse et al.[21]
H3 primer R	ATTGCRCCRAATATGCCTCTAGT	40	
H3 sonda	CAGGATCACATATGGGSCCTGTCCCAG	10	
E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	5	Corman et al.[22]
E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	5	
E_Sarbeco_P1	ACACTAGCCATCCTTACTGCGCTTCG	5	
VTB2-HAdVCf	GAGACGTACTTCAGCCTGAAT	25	Wolf et al.[17]
VTB2-HAdVCr	GATGAACCGCAGCGTCAA	25	
VTB2-HAdVC probe	CCTACGCACGACGTGACCACAGA	15	

TABLE 2 Number of positive samples for other respiratory viruses and their percentages relative to SARS-CoV-2 positivity status

Virus	SARS-CoV-2 positive samples			SARS-CoV-2 negative samples		
	Tested	Detected	Percent	Tested	Detected	Percent
Influenza A	418	6	1.43	569	10	1.75
Influenza B	418	0	0.00	569	0	0.00
Adenovirus	418	18	4.30	569	49	8.61
Rhinovirus 1/2[a]	67	18	26.8	210	55	26.2
Rhinovirus 2/2[a]	73	9	12.3	199	28	14.1
Enterovirus 68	20	0	0.00	56	0	0.00

[a]Names of the primer-probe kit according to the manufacturer of the commercial kit used.

Of the 987 samples included in this study, all of them were analyzed for SARS-CoV-2, FLUAVs, FLUBV and HAdV-C. For RV 1/2, 277 were analyzed, 272 for RV 2/2 and 76 for EV-68. A total of 418 (42.3%) samples were positive for SARS-CoV-2, 16 (1.62%) samples were positive for FLUAV, 15 with pdmInfA primer and one with pdmH1, no sample was positive for FLUBV or EV-68 and 67 (6.78%) samples were positive for HAdV-C. Seventy-three samples were positive for RV 1/2 (26.3%) and 37 for RV 2/2 (13.6%), these results can be seen in Table 2 and Figure 2. Figure 4 shows positive cases for all respiratory viruses tested distributed throughout the year.

In addition, some coinfections were also detected. SARS-CoV-2 was detected in coinfections with all the other three viruses found in this study including a triple coinfection with FLUAV and HAdV-C. Also, RV and HAdV-C were the viruses most found in coinfections with SARS-CoV-2. Viral combinations in coinfections and their frequencies can be more clearly visualized in Figure 3.

4 | DISCUSSION

This past year had several unusual aspects due to the arrival of SARS-CoV-2 all around the world, Brazil's first case was in February, and in March several states already had cases, including Rio Grande do Sul state where this study took place.[23] One of the peculiar aspects of COVID-19 pandemic was the preventive measures taken to avoid infection, like face masking by populations that usually did not have this habit, the isolation of people presenting respiratory symptoms, frequent hand washing, and social distancing, therefore these precautions probably had an effect in other viruses besides SARS-CoV-2.[16,24,25]

In this study, it was possible to verify a low frequency of FLUAV in hospitalized patients with cases of ARDS during the COVID-19 pandemic. Only 1.62% of the samples analyzed were positive for this virus even if FLUAV is an important cause of ARDS during autumn/winter months mainly in elderly people that, in fact, compose most of the patients included in this study.[26-29] For comparison, in 2019, about 17.8% (5.714) of 32.048 ARDS' samples were attributed to one of FLUVs throughout Brazil.[30] One probable explanation for this is the vaccination status of the population, which is above 90% coverage in the state.[31] HAdV-C also presented a low frequency (6.78%) which was also described in similar studies during 2020 for other respiratory viruses[32] and in the case of RV, it remains present at a considerable rate (~20%) in comparison to the past years,[33] even though being higher than that find by Nowak et al.[32] Notwithstanding, in this study, a smaller number of samples were analyzed for RV.

About the distribution of cases over time, in Figure 4 it is possible to evaluate that after a few weeks under the orange flag of the controlled distancing system of the state government that indicates a medium risk of contagion,[13,14] the positivity of SARS-CoV-2 rose.

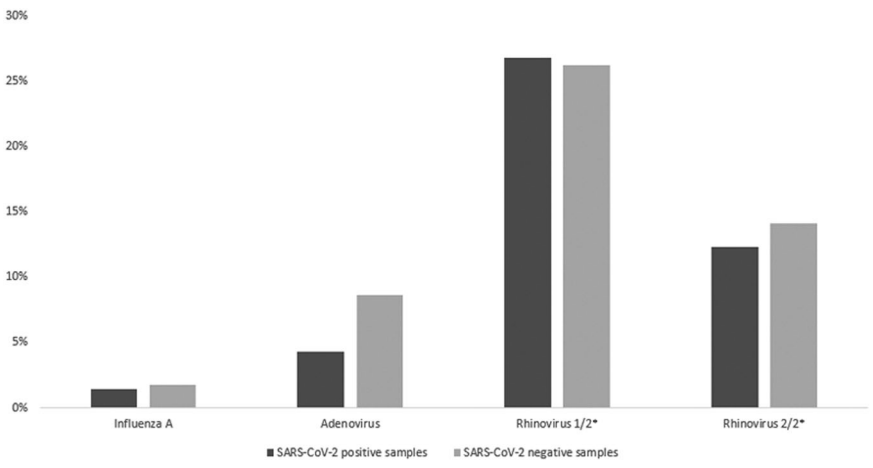

FIGURE 2 Frequencies Influenza A, adenovirus and rhinovirus (RV) positive samples distributed in the 418 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive or the 569 negative cases. *Names of the primer-probe kit according to the manufacturer of the commercial kit used. Fewer samples were tested for RV

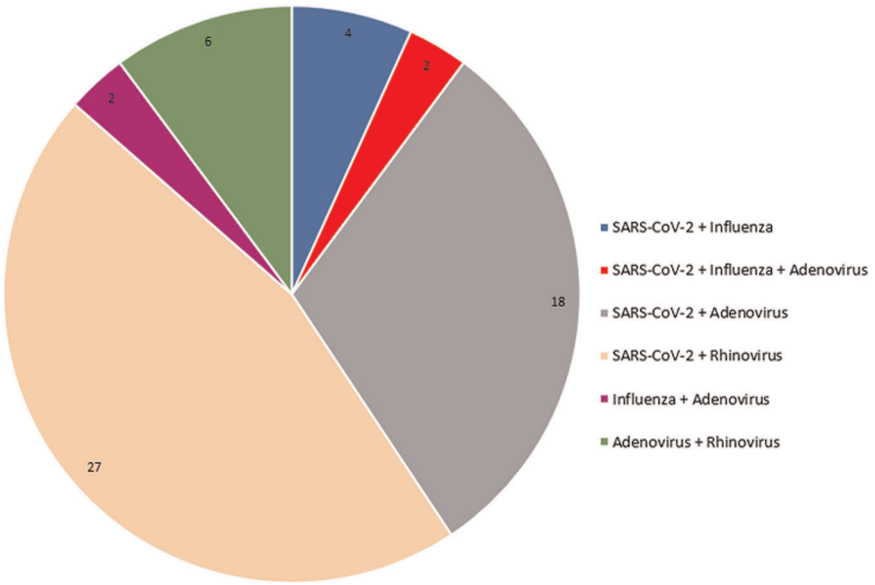

FIGURE 3 Coinfections cases between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Influenza A (FLUAV), adenovirus (HAdV) and rhinovirus. Different dual coinfections were found as well as a triple coinfection with SARS-CoV-2, FLUAV and HadV

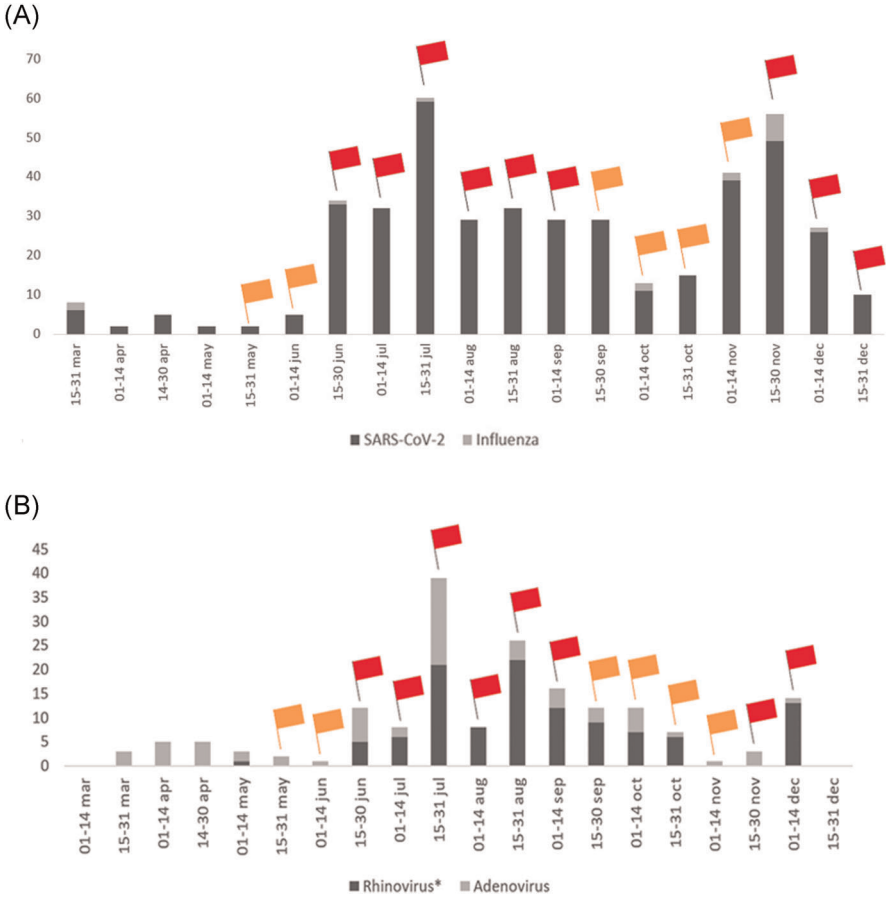

FIGURE 4 Distribution of positive cases along the year and current risk flag of the government for certain measures of social distancing. The red one being a high risk of contagion which imposes more restrictive measures on businesses and establishments and the orange, moderate risk, with more flexible measures. (A) Distribution of 418 SARS-CoV-2 and 16 influenza A positive patients. (B) Positive cases of adenovirus and rhinovirus (RV) over the year. *Fewer samples were tested for RV and so distribution may be biased

After the second and longer period with orange flags, around eight weeks, most of the FLUAV positive cases of this study occurred in November accompanied by an increase also in the number of positive SARS-CoV-2 cases, probably because people may have circulated more due to the orange flag that must have conveyed a false sense of safety to the population.

We have detected some viral coinfections including SARS-CoV-2 as presented in Table 2. SARS-CoV-2 + HAdV-C and SARS-CoV-2 + RV coinfections occurred more often probably due to the greater frequency of these viruses in samples tested, and in spite of low FLUAV frequency, a dual and a triple coinfection with this virus were detected. Viral respiratory coinfections are very common and several studies already

reported viral coinfections with SARS-CoV-2, including with FLUAV, although it is probably not occurring as often as it might be expected, which may be due to a lack of diagnosis for other viruses in this pandemic period.[32,34-39]

Still, on the topic of coinfections, there is a risk of disease exacerbation in these cases, considering that the immunologic system is already disturbed due to the first infection. In studies that reported viral coinfections with COVID-19, it is not yet clear if these cases are associated with more severe disease,[38,40-42] except for the study of Yue et al.[43] that suggested that SARS-CoV-2 and FLUBV coinfection might be associated with more severe cases, but the authors commented that this virus was circulating more than FLUAV so this finding may be biased. Another exception is the work of Li et al.[44] which reported that coinfections with COVID-19 are more associated with patients in the intensive care unit but did not separate viral from bacterial coinfections. In sum, the understanding of possible COVID-19 aggravation by coinfection with other respiratory viruses is still largely unknown, and in this study, we did not aim to evaluate clinical outcomes, so this topic still needs further investigation in future studies.

5 | CONCLUSION

In the present work, FLUV was reported in a very low frequency among hospitalized patients with ARDS compared to the past years, probably due to preventive measures taken to avoid COVID-19 and the high influenza vaccination coverage in 2020. We also demonstrated a relatively normal frequency of RV. Some viral coinfections were detected, including SARS-CoV-2 and FLUAV and a triple coinfection of SARS-CoV-2, FLUAV, and HAdV-C, although it is still not clear if viral coinfection is associated with more severe disease.

ACKNOWLEDGMENTS

We would like to thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for the graduate scholarships, Financiadora de Estudos e Projetos – Finep and RedeVírus Ministério da Ciência, Tecnologia, Inovações e Comunicações – MCTIC for the research funding. This study is part of the research efforts from Rede Corona-ômica BR MCTI/FINEP affiliated to RedeVírus/MCTI (FINEP = 01.20.0029.000462/20, CNPq = 404096/2020-4).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHORS CONTRIBUTIONS

Ana Karolina Antunes Eisen: *Sample selection*, *extraction*, *analysis*, *result analysis*, *writing*. Juliana Schons Gularte: *Extraction*, *analysis*, *language review*. Meriane Demoliner: *Analysis*, *figure elaboration*. Vyctoria Malayhka de Abreu Goés Pereira: *Sample selection*, *analysis*. Fágner Henrique Heldt: *Extraction*, *figure elaboration*. Micheli Filippi: *Sample selection*, *analysis*. Paula Rodrigues de Almeida: *Textual review*. Alana Witt Hansen: *Sample selection*, *result analysis*. Juliane Deise Fleck: *Textual review*. Fernando Rosado Spilki: *Study planning*, *textual review*.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmv.26975

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ana Karolina Antunes Eisen http://orcid.org/0000-0003-0144-1695
Juliana Schons Gularte https://orcid.org/0000-0003-1822-147X
Meriane Demoliner http://orcid.org/0000-0002-7160-7649
Vyctoria Malayhka de Abreu Goés Pereira https://orcid.org/0000-0002-4833-4400
Fágner Henrique Heldt https://orcid.org/0000-0002-6164-3312
Micheli Filippi https://orcid.org/0000-0003-3328-2326
Paula Rodrigues de Almeida https://orcid.org/0000-0002-4108-8796
Alana Witt Hansen https://orcid.org/0000-0001-8728-8569
Juliane Deise Fleck https://orcid.org/0000-0002-3523-3192
Fernando Rosado Spilki https://orcid.org/0000-0001-5804-7045

REFERENCES

1. ICTV. Naming the 2019 Coronavirus. 2020. https://talk.ictvonline.org/. Accessed January 9, 2021.
2. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579(7798):265-269. https://doi.org/10.1038/s41586-020-2008-3
3. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273. https://doi.org/10.1038/s41586-020-2012-7
4. WHO. COVID-19 WHO Dashboard. 2020. https://www.covid19.who.int/. Accessed January 9, 2021.
5. WHO. Archived: WHO Timeline – COVID-19. World Health Organization. 2020. https://www.who.int/news-room/detail/27-04-2020-who-timeline—covid-19. Accessed July 30, 2020.
6. Feikin DR, Njenga MK, Bigogo G, et al. Etiology and incidence of viral and bacterial acute respiratory illness among older children and adults in rural western kenya, 2007-2010. *PLoS One*. 2012;7(8):2007-2010. https://doi.org/10.1371/journal.pone.0043656
7. Seo YB, Song JY, Choi MJ, et al. Etiology and clinical outcomes of acute respiratory virus infection in hospitalized adults. *Infect Chemother*. 2014;46(2):67-76. https://doi.org/10.3947/ic.2014.46.2.67
8. Bezerra PGM, Britto MCA, Correia JB, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years. *PLOS One*. 2011;6(4):4-12. https://doi.org/10.1371/journal.pone.0018928
9. Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C. Acute respiratory infection and influenza-like illness viral etiologies in Brazilian adults.pdf. *J Med Virol*. 2008;80:1824-1827. https://doi.org/10.1002/jmv.21295
10. Gregianini TS, Varella IRS, Fisch P, Martins LG, Veiga ABG. Dual and triple infections with influenza A and B viruses: a case-control study in southern Brazil. *J Infect Dis*. 2019;220(6):961-968. https://doi.org/10.1093/infdis/jiz221
11. Straliotto SM, Siqueira MM, Muller RL, Fischer GB, Cunha MLT, Nestor SM. Viral etiology of acute respiratory infections among children in Potto Alegre, RS, Brazil. *Rev Soc Bras Med Trop*. 2002;35(4):283-291. https://doi.org/10.1590/s0037-86822002000400002

12. Girardi V, Gregianini TS, Gularte JS, et al. Temporal dynamics of Human mastadenovirus species in cases of respiratory illness in southern Brazil. *Brazil J Microbiol*. 2019;50:677-684.
13. GOV-RS. Modelo de Distanciamento Controlado Rio Grande do Sul. Governo do Estado do Rio Grande do Sul. Published 2020. https://distanciamentocontrolado.rs.gov.br/. Accessed January 12, 2021.
14. GOV-RS. DECRETO No 55.241, DE 10 DE MAIO DE 2020. Estado do Rio Grande do Sul Assembléia Legislativa. 2020. http://www.al.rs.gov.br/legis/M010/M0100099.ASP?Hid_Tipo=TEXTO&Hid_TodasNormas=66394&hTexto==&Hid_IDNorma=66394
15. Leung NHL, Chu DKW, Shiu EYC, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med*. 2020;26:676-680. https://doi.org/10.1038/s41591-020-0843-2
16. Wu D, Lu J, Liu Y, Zhang Z, Luo L. Positive effects of COVID-19 control measures on influenza prevention. *Int J Infect Dis*. 2020;95:345-346. https://doi.org/10.1016/j.ijid.2020.04.009
17. Wolf S, Hewitt J, Greening GE. Viral multiplex quantitative PCR assays for tracking sources of fecal contamination. *Appl Environ Microbiol*. 2010;76(5):1388-1394. https://doi.org/10.1128/AEM.02249-09
18. WHO. *CDC protocol of realtime RTPCR for swine influenza A (H1N1)*. World Health Organization; 2009.
19. Selvaraju SB, Selvarangan R. Evaluation of Three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses. *J Clin Microbiol*. 2010;48(11):3870-3875. https://doi.org/10.1128/JCM.02464-09
20. Rönkkö E, Ikonen N, Kontio M, Ziegler T. Validation and diagnostic application of NS and HA gene-specific real-time reverse transcription-PCR assays for detection of 2009 pandemic influenza A (H1N1) viruses in clinical specimens. *J Clin Microbiol*. 2011;49(5):2009-2011. https://doi.org/10.1128/JCM.00259-11
21. Tse H, To KKW, Wen X, et al. Clinical and virological factors associated with viremia in pandemic influenza A/H1N1/2009 virus infection. *PLOS One*. 2011;6(9):e22534. https://doi.org/10.1371/journal.pone.0022534
22. Corman V, Bleicker T, Brunink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR. *Eurosurveillance*. 2020;25(3). https://doi.org/10.2807/1560-7917.es.2020.25.3.2000045
23. MS - Ministério da Saúde. Painel Coronavírus. 2020. Published 2020. https://covid.saude.gov.br/. Accessed January 12, 2021.
24. Hirotsu Y, Maejima M, Shibusawa M, Amemiya K, Nagakubo Y. Analysis of Covid-19 and non-Covid-19 viruses, including influenza viruses, to determine the influence of intensive preventive measures in Japan. *J Clin Virol*. 2020. https://doi.org/10.1016/j.jcv.2020.104543
25. Chotpitayasunondh T, Kølsen T, Heraud J, et al. Influenza and COVID-19. What does co-existence mean?. *Influenza Other Respir Viruses* 2020:1-6. https://doi.org/10.1111/irv.12824
26. Zhang D, He Z, Xu L, et al. Epidemiology characteristics of respiratory viruses found in children and adults with respiratory tract infections in southern China. *Int J Infect Dis*. 2014;25:159-164. https://doi.org/10.1016/j.ijid.2014.02.019
27. Yu J, Xie Z, Zhang T, et al. Comparison of the prevalence of respiratory viruses in patients with acute respiratory infections at different hospital settings in North China, 2012-2015. *BMC Infect Dis*. 2018;18(1):1-10. https://doi.org/10.1186/s12879-018-2982-3
28. Sakamoto H, Ishikane M, Ueda P. Seasonal Influenza Activity During the SARS-CoV-2 Outbreak in Japan. *JAMA*. 2020;323(19):1769-1971. https://doi.org/10.1001/jama.2020.6173
29. Liu T, Li Z, Zhang S, et al. Viral Etiology of acute respiratory tract infections in hospitalized children and adults in Shandong Province, China. *Virol J*. 2015:1-7. https://doi.org/10.1186/s12985-015-0388-z
30. MS. Influenza: Monitoramento até a Semana Epidemiológica 49 de 2019. Ministério da Saúde - Boletim Epidemiológico 38. 2019. https://portalarquivos.saude.gov.br/images/pdf/2019/dezembro/20/Boletim-epidemiologico-SVS-38-2-interativo.pdf. Accessed January 12, 2021.
31. MS - Ministério da Saúde. Campanha Nacional de Vacinação Contra Influenza 2020 VACINÔMETRO. SIPNI - Sistema de Informações do Programa Nacional de Imunizações. 2020. http://sipni-gestao.datasus.gov.br/si-pni-web/faces/relatorio/consolidado/vacinometroInfluenza.jsf. Accessed January 13, 2021.
32. Nowak MD, Sordillo EM, Gitman MR, Mondolfi AEP. Co-infection in SARS-CoV-2 infected patients: Where are influenza virus and rhinovirus/enterovirus? *J Med Virol*. 2020;92(10):1699-1700. https://doi.org/10.1002/jmv.25953
33. Kurskaya O, Ryabichenko T, Leonova N, et al. Viral etiology of acute respiratory infections in hospitalized children in Novosibirsk City, Russia (2013 – 2017). *PLOS One*. 2018;13:e0200117.
34. Zhu X, Ge Y, Wu T, et al. Co-infection with respiratory pathogens among COVID-2019 cases. *Virus Res*. 2020;285:198005. https://doi.org/10.1016/j.virusres.2020.198005
35. D'Abramo A, Lepore L, Palazzolo C, et al. Acute respiratory distress syndrome due to SARS-CoV-2 and Influenza A co-infection in an Italian patient: Mini-review of the literature. *Int J Infect Dis*. 2020;(97):1201-9712. https://doi.org/10.1016/j.ijid.2020.06.056
36. Zheng X, Wang H, Su Z, et al. Co-infection of SARS-CoV-2 and Influenza virus in Early Stage of the COVID-19 Epidemic in Wuhan, China. *J Infect*. 2020;81(2):19-21. https://doi.org/10.1016/j.jinf.2020.05.041
37. Huang B-R, Lin Y-L, Wan C-K, et al. Co-infection of influenza B virus and SARS-CoV-2: a case report from Taiwan. *J Microbiol Immunol Infect*. 2020. https://doi.org/10.1016/j.jmii.2020.06.011
38. Khodamoradi Z, Moghadami M, Lotfi M. Co-infection of coronavirus disease 2019 and influenza a: A report from Iran. *Arch Iran Med*. 2020;23(4):239-243. https://doi.org/10.34172/aim.2020.04
39. Antony SJ, Almaghlouth NK, Heydemann EL. Are co-infections with COVID-19 and Influenza low or underreported? An observational study examining current published literature including three new unpublished cases. *J Med Virol*. 2020;92(11):2489-2497. https://doi.org/10.1002/jmv.26167
40. Kondo Y, Miyazaki S, Yamashita R, Ikeda T. Coinfection of SARS-CoV-2 and influenza A virus. *BMJ*. 2020;13(e236812):2-4. https://doi.org/10.1136/bcr-2020-236812
41. Cuadrado-Payán E, Montagud-Marrahi E, Torres-Elorza M, et al. SARS-CoV-2 and influenza virus co-infection. *Lancet*. 2020;395:e84. https://doi.org/10.1016/S0140-6736(20)31052-7
42. Konala VM, Adapa S, Gayam V, Naramala S, Daggubati SR. Co-infection with influenza A and COVID-19. *BMC Infect Dis*. 2020:2018-2021. https://doi.org/10.12890/2020
43. Yue H, Zhang M, Xing L, et al. The epidemiology and clinical characteristics of co-infection of SARS-CoV-2 and influenza viruses in patients during COVID-19 outbreak. *J Med Virol*. 2020;92(11):2870-2873. https://doi.org/10.1002/jmv.26163
44. Li Z, Chen Z, Chen L-D, et al. Coinfection with SARS-CoV-2 and other respiratory pathogens in patients with COVID-19 in Guangzhou, China. *J Med Virol*. 2020;92(22):2381-2383. https://doi.org/10.1002/jmv.26073

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Eisen AKA, Gularte JS, Demoliner M, et al. Low circulation of Influenza A and coinfection with SARS-CoV-2 among other respiratory viruses during the COVID-19 pandemic in a region of southern Brazil. *J Med Virol*. 2021;93:4392–4398. https://doi.org/10.1002/jmv.26975