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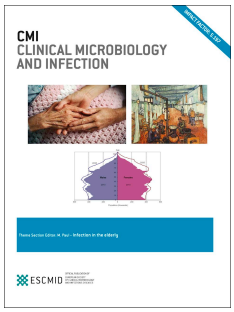

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Molecular detection of SARS-CoV-2 and other respiratory viruses in saliva and classroom air: a two winters tale

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Abstract

Objectives: To compare the prevalence of SARS-CoV-2 and other respiratory viruses in saliva and bioaerosols between two winters and model the probability of virus detection in classroom air for different viruses.

Methods: We analyze saliva, air, and air cleaner filter samples from studies conducted in two Swiss secondary schools (age 14-17 years) over seven weeks during the winters of 2021/22 and 2022/23. Two bioaerosol sampling devices and HEPA filters from air cleaners were used to collect airborne virus particles in five classrooms. Daily bioaerosol samples were pooled for each sampling device before PCR analysis of a panel of 19 respiratory viruses and viral subtypes. The probability of detection of airborne viruses was modelled using an adjusted Bayesian logistic regression model.

Results: Three classes (58 students) participated in 2021/22, and two classes (38 students) in 2022/23. During winter 2021/22, SARS-CoV-2 dominated in saliva (19 of 21 positive samples) and bioaerosols (9 of 10). One year later, there were 50 positive saliva samples, mostly influenza B, rhinovirus, and adenovirus, and two positive bioaerosol samples, one rhinovirus and one adenovirus. The weekly probability of airborne detection was 34% (95%-credible interval [CrI] 22%−47%) for SARS-CoV-2 and 10% (95%-CrI 5%-16%) for other respiratory viruses.

Conclusions: There was a distinct shift in the distribution of respiratory viruses from SARS-CoV-2 during the Omicron wave to other respiratory viruses one year later. SARS-CoV-2 is more likely to be detected in the air than other endemic respiratory viruses, possibly reflecting differences in viral characteristics and the composition of virus-carrying particles that facilitate airborne long-range transmission.

Keywords: respiratory viruses; SARS-CoV-2; influenza; airborne transmission; molecular detection

Introduction

The transmission of respiratory viruses, such as SARS-CoV-2 and influenza, in schools and other indoor environments is difficult to control [1]. During the COVID-19 pandemic, non-pharmaceutical interventions and physical distancing reduced the spread of SARS-CoV-2 and other seasonal respiratory viruses, but a resurgence of respiratory infections followed the relaxation of these measures [2–4]. Following epidemic peaks, a shift in the circulation of respiratory viruses occurs [5], which can be identified by frequent collection of non-invasive saliva samples [6].

Respiratory viruses spread via multiple routes, including respiratory particles such as large droplets and small aerosols. Unlike larger droplets, which settle quickly, aerosols can remain suspended in the air for extended periods [7]. Airborne infectious pathogens are primarily found in smaller particles and the distribution is similar across various pathogens [8]. Thus, pathogen-carrying aerosols have the potential for long-range transmission, but the larger concentration of particles near the infectious person favors short-range transmission [7].

We compared saliva samples, bioaerosol samples, and samples from the HEPA-filters of air cleaners that were collected as part of two studies conducted in a Swiss school setting in winter 2021/22 (during the SARS-CoV-2 omicron wave) [9] and winter 2022/23 [10].

Methods

Data were collected in two secondary schools (students age 14-17 years) in the canton of Solothurn, Switzerland, during a seven-week study period from the end of January to the beginning of March. Three classes (two classrooms) participated in 2021/22 and two classes (two classrooms) in 2022/23. An air quality device (AQ Guard, Palas GmbH, Karlsruhe, Germany) continuously measured indoor CO_2 levels, temperature, and humidity. A detailed comparison of the study settings can be found in Appendix Table S1.

Testing for a panel of respiratory infections was performed weekly in 2021/22 and bi-weekly in 2022/23 using saliva collection kits with saline solution. Airborne respiratory viruses were collected in each classroom with a cyclonic bioaerosol sampling device (Coriolis Micro Air, Bertin

Instruments Montigny-le-Bretonneux, France) and the BioSpot-VIVAS condensation particle growth collection device (Aerosol Devices Inc., Ft. Collins, CO, USA) [11]. The HEPA filters from the portable air cleaner (Xiaomi Mi Air Pro 70m2, Shenzhen, China) were removed and divided into 20 fields. For each field, one swab moistened with sterile Phosphate-Buffered Saline was collected, amounting to a total of 20 swabs per filter. Saliva and airborne samples were transported to the laboratory on the same day and stored immediately at −80°C until further processing [12]. Before real-time (RT)-PCR analysis, daily bioaerosol samples were pooled for each sampling device and enriched using Amicon Ultra-15 Centrifugal filters as described previously [9]. Saliva samples were analyzed directly without prior filtration/enrichment. The Allplex RV Master Assay (Seegene, Seoul, South Korea) detects a panel of 19 major respiratory viruses and viral subtypes, including SARS-CoV-2, influenza A/B virus, respiratory syncytial virus, metapneumovirus, adenovirus, rhinovirus, and parainfluenza virus. The technical study protocols were identical in both study periods.

We used descriptive statistics to present differences in the type and number of respiratory viruses detected in saliva and airborne samples between 2021/22 and 2022/23. A Bayesian logistic regression model was used to estimate the probability of detecting any SARS-CoV-2 versus non-SARS-CoV-2 viruses in the air during a study week, adjusting for differences in the study settings, whether a positive saliva sample was found in the same week, the interventions implemented during the studies (compulsory face mask wearing and portable air cleaners), and the daily maximum CO_2 levels (as a proxy for indoor air quality and ventilation). Appendix Text A provides a detailed model description. All analyses were done in R version 4.3.2 and Bayesian modeling was performed using the probabilistic programming language Stan version 2.26.1.

The Ethics Committee of the canton of Bern, Switzerland, approved the study (reference no. 2021–02377). For the saliva samples, we included all students who were willing to participate and obtained written informed consent from their caregivers.

Results

In 2021/22, 51 of 58 students (84%) participated in weekly saliva testing. There were 21 positive saliva

samples during the study, 19 SARS-CoV-2, one influenza A virus, and one adenovirus (Figure 1a, left). There were 10 positive bioaerosol samples, nine SARS-CoV-2 and one adenovirus. There were eight positive samples on the HEPA-filters, six SARS-CoV-2, one influenza A virus and one adenovirus. In 2022/23, 37 of 38 students (97%) participated in bi-weekly saliva testing. There were 50 positive saliva samples, mostly influenza B virus, rhinovirus, and adenovirus (Figure 1a, right). There were two positive bioaerosol samples, one rhinovirus and one adenovirus. There were four positive samples on the HEPA-filters of the air cleaners, one influenza B virus, one rhinovirus, one adenovirus, and one SARS-CoV-2. Overall, we found six positive air-saliva samples of the same virus in the same classroom in the same week (four SARS-CoV-2 and two non-SARS-CoV-2 viruses; Figure 1b), suggesting they were paired samples. In saliva, Ct values were significantly lower for SARS-CoV-2 than other respiratory viruses ($\Delta -2.45$, p=0.02; Appendix Figure S1).

SARS-CoV-2 was more likely detected in bioaerosols than other respiratory viruses (posterior probability 97%, adjusted odds ratio 4.8, 95%-CrI 2.6−9.0). The probability of airborne molecular detection was 34% (95%-credible interval [CrI] 22%−47%) for SARS-CoV-2 versus 10% (95%-CrI 5%-16%) for non-SARS-CoV-2 viruses (Figure 1c). We adjusted estimates for differences in maximum daily CO_2, which increased from 1,134 ppm (standard deviation [SD] 277 ppm) in 2021/22 to 2,224 ppm (SD 321 ppm) in 2022/23. Relative humidity (38% [SD 6%] in 2021/22 vs. 38% [SD 5%] in 2022/23) and temperature (19°C [SD 2°C] in 2021/22 vs. 22°C [SD 1°C in 2022/23] were similar at around 38% and 20°C, respectively.

122

123 **Fig 1.** Comparison of molecular detection of respiratory viruses between winter 2021/22 and winter
124 2022/23. **(a)** Distribution of respiratory viruses found in saliva. IF: influenza A/B virus, HRV: human
125 rhinovirus, AdV: adenovirus, CoV: SARS-CoV-2, MPV: human metapneumovirus, PIV: parainfluenza virus.
126 **(b)** Positive samples in saliva and bioaerosols per study week. **(c)** Probability of detecting any SARS-CoV-2
127 and non-SARS-CoV-2 viruses in bioaerosols during a study week (posterior mean as dots, interquartile range
128 as box, 95%-CrI as error bars), with the posterior probability that airborne detection was more frequent for
129 SARS-CoV-2 than non-SARS-CoV-2 shown on top.

Discussion

We compared the molecular detection of respiratory viruses in saliva, air, and filter samples collected in two studies in Swiss secondary schools in the winter seasons of 2021/22 and 2022/23. In winter 2021/22, we predominantly identified SARS-CoV-2 in saliva, air, and air filter samples. Conversely, in 2022/23, we primarily detected non-SARS-CoV-2 viruses, such as influenza viruses and adenoviruses, in saliva samples, but these were rarely found in air or filter samples.

Overall, the likelihood of molecular airborne detection was substantially higher for SARS-CoV-2 compared to non-SARS-CoV-2 viruses, even when we adjusted for covariates and differences between the studies. Although the molecular assay used has not been formally validated for respiratory viruses other than SARS-CoV-2 in saliva samples, this sample type is increasingly replacing more invasive nasopharyngeal swabs in surveillance settings and may have comparable performance [13]. Besides differences in virus circulation in the population during the study periods, a plausible explanation is that SARS-CoV-2 can remain airborne for extended durations, thus facilitating long-range transmission, matching the observation of superspreading events during the pandemic. This contrasts with other respiratory viruses, where airborne detection was found to be infrequent in our studies. Therefore, prolonged close contact may be relatively more important for transmission of respiratory viruses other than SARS-CoV-2, although close contact also facilitates transmission of SARS-CoV-2 [1,14].

Technical factors are unlikely to account for the differences in airborne detection. The two studies employed identical bioaerosol samplers and laboratory methods, and no technical problems occurred. Temperature and relative humidity were also similar. Ventilation changed, with higher CO_2 levels in 2022/23 potentially enhancing airborne survival, but this and other differences were controlled for in the statistical analysis. Therefore, it is plausible that the difference in airborne detection may be due to differences in virus characteristics, particularly between SARS-CoV-2 and non-SARS-CoV-2 viruses, which may influence the distribution and survival of virus in airborne particles of different sizes[7]. Non-SARS-CoV-2 respiratory virus infections may result in smaller amounts of exhaled

bioaerosols, falling below the detection limit of current sampling devices [15]. Interestingly, we found higher Ct values for non-SARS-CoV-2 saliva samples, suggesting lower viral loads. However, this finding must be interpreted with caution because Ct values (or viral loads) can be highly variable due to sampling techniques and biological differences, and higher viral loads may not necessarily translate into increased infectiousness [16]. Finally, other non-SARS CoV-2 human coronaviruses, such as HCoV-OC43, and emerging respiratory viruses may exhibit different behaviors that warrant additional study.

Other unobserved factors could also explain differences in airborne detection, such as the more frequent presence of highly infectious students (superspreaders) with SARS-CoV-2 in the classroom in winter 2021/22, who could have emitted more bioaerosols. Differences in host immunity may also have played a role, although SARS-CoV-2 was primarily detected in saliva and air samples in winter 2021/22, when students were likely to have higher immunity (recently vaccinated or recently recovered students) compared with winter 2022/23, which may indicate less airborne spread of SARS-CoV-2 and lower susceptibility to SARS-CoV-2 in winter 2021/22. Prior immunity to other respiratory viruses has not been measured, but vaccination is typically used less frequently to prevent non-SARS-CoV-2 respiratory viruses.

In conclusion, we observed a distinct shift in the distribution of respiratory viruses from SARS-CoV-2 in the winter of 2021/22 to non-SARS-CoV-2 viruses in 2022/23, reflecting the transition from epidemic to endemic transmission of SARS-CoV-2. Molecular detection of airborne SARS-CoV-2 was more frequent than other endemic respiratory viruses. Future studies should investigate the seasonality of SARS-CoV-2 and non-SARS-CoV-2 respiratory viruses and the contribution of close contact versus airborne long-range transmission to overall transmission of respiratory infections in congregate indoor settings.

Conflicts of Interest

All authors have declared that no competing interests exist.

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Author contributions

Conception and design: NB, KZ, LF, PB, PJ, TS. Epidemiological and environmental data collection: NB, PJ, KZ, TS, LF. Laboratory data collection: PB, LFu. Statistical analysis: NB. Paper draft: NB, ME, LF. All authors reviewed and approved the final version of the manuscript.

References

1. Leung NHL, Chu DKW, Shiu EYC, Chan K-H, McDevitt JJ, Hau BJP, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. Nat Med. 2020;26: 676–680. doi:10.1038/s41591-020-0843-2

2. Poole S, Brendish NJ, Tanner AR, Clark TW. Physical distancing in schools for SARS-CoV-2 and the resurgence of rhinovirus. Lancet Respir Med. 2020;8: e92–e93. doi:10.1016/S2213-2600(20)30502-6

3. Sauteur PMM, Beeton ML, Uldum SA, Bossuyt N, Vermeulen M, Loens K, et al. Mycoplasma pneumoniae detections before and during the COVID-19 pandemic: results of a global survey, 2017 to 2021. Eurosurveillance. 2022;27: 2100746. doi:10.2807/1560-7917.ES.2022.27.19.2100746

4. Kandeel A, Fahim M, Deghedy O, Roshdy WH, Khalifa MK, Shesheny RE, et al. Resurgence of influenza and respiratory syncytial virus in Egypt following two years of decline during the COVID-19 pandemic: outpatient clinic survey of infants and children, October 2022. BMC Public Health. 2023;23: 1067. doi:10.1186/s12889-023-15880-9

5. Pierangeli A, Scagnolari C, Selvaggi C, Monteleone K, Verzaro S, Nenna R, et al. Virological and clinical characterization of respiratory infections in children attending an emergency department during the first autumn–winter circulation of pandemic A (H1N1) 2009 influenza virus. Clin Microbiol Infect. 2012;18: 366–373. doi:10.1111/j.1469-0691.2011.03590.x

6. Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, et al. Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2021;27: 285.e1-285.e4. doi:10.1016/j.cmi.2020.05.001

7. Wang CC, Prather KA, Sznitman J, Jimenez JL, Lakdawala SS, Tufekci Z, et al. Airborne transmission of respiratory viruses. Science. 2021;373: eabd9149. doi:10.1126/science.abd9149

8. Fennelly KP. Particle sizes of infectious aerosols: Implications for infection control. Lancet Respir Med. 2020;8: 914–924. doi:10.1016/S2213-2600(20)30323-4

9. Banholzer N, Zürcher K, Jent P, Bittel P, Furrer L, Egger M, et al. SARS-CoV-2 transmission with and without mask wearing or air cleaners in schools in Switzerland: A modeling study of epidemiological, environmental, and molecular data. PLOS Med. 2023;20: e1004226. doi:10.1371/journal.pmed.1004226

10. Banholzer N, Jent P, Bittel P, Zürcher K, Furrer L, Bertschinger S, et al. Air cleaners and respiratory infections in schools: A modeling study using epidemiological, environmental, and molecular data. medRxiv; 2023. doi:10.1101/2023.12.29.23300635

11. Lednicky J, Pan M, Loeb J, Hsieh H, Eiguren-Fernandez A, Hering S, et al. Highly efficient collection of infectious pandemic influenza H1N1 virus (2009) through laminar-flow water based condensation. Aerosol Sci Technol. 2016;50: i–iv. doi:10.1080/02786826.2016.1179254

12. Huber M, Schreiber PW, Scheier T, Audigé A, Buonomano R, Rudiger A, et al. High efficacy of saliva in detecting SARS-CoV-2 by RT-PCR in adults and children. Microorganisms. 2021;9: 642. doi:10.3390/microorganisms9030642

13. To KKW, Yip CCY, Lai CYW, Wong CKH, Ho DTY, Pang PKP, et al. Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: A diagnostic validity study. Clin Microbiol Infect. 2019;25: 372–378. doi:10.1016/j.cmi.2018.06.009

14. Lind ML, Dorion M, Houde AJ, Lansing M, Lapidus S, Thomas R, et al. Evidence of leaky protection following COVID-19 vaccination and SARS-CoV-2 infection in an incarcerated population. Nat Commun. 2023;14: 5055. doi:10.1038/s41467-023-40750-8

15. Belser JA, Pulit-Penaloza JA, Maines TR. Aerosolize this: Generation, collection, and analysis of aerosolized virus in laboratory settings. PLOS Pathog. 2023;19: e1011178. doi:10.1371/journal.ppat.1011178

16. Jones TC, Biele G, Mühlemann B, Veith T, Schneider J, Beheim-Schwarzbach J, et al. Estimating infectiousness throughout SARS-CoV-2 infection course. Science. 2021;373: eabi5273. doi:10.1126/science.abi5273