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Molecular epidemiology of respiratory syncytial virus in Switzerland 2019–2024 from nucleic acid testing and whole-genome sequencing

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Summary

BACKGROUND AND AIMS: Respiratory syncytial virus (RSV) infection is one of the leading causes of hospitalisation in infants, the elderly and immunocompromised patients, with significant morbidity and mortality rates. Despite its global impact, epidemiological surveillance of RSV in Switzerland has historically been limited compared to the USA and European countries. A significant surge in RSV activity and hospitalisations after the COVID-19 pandemic, along with the introduction of new monoclonal antibodies and RSV vaccines, has led to an increased interest in the molecular epidemiology of RSV. To ensure continued efficacy of the new preventive options, monitoring of the genetic diversity of circulating RSV strains, their evolutionary dynamics and the potential emergence of resistance mutations is of central importance. The present study aimed to characterise the genetic diversity and seasonal trends of RSV dynamics in Switzerland from 2019 to 2024, mainly over the last two post-pandemic winter seasons (2022/23 and 2023/24).

METHODS: A total of 48,897 respiratory clinical specimens from 30,782 patients with respiratory tract infections were tested for RSV at a tertiary care centre in Northwestern Switzerland between July 2019 and June 2024. RSV activity over these seasons was investigated. Amplicon-based whole-genome sequencing was performed on 182 selected samples, with 125 high-quality consensus genomes and phylogenies reconstructed. Lineage distribution and seasonal subtype prevalence were compared to European data.

RESULTS: RSV activity was absent during the 2020/21 pandemic season, but surged with an off-season peak in summer 2021. RSV B predominated during the 2022/23 season, while a shift to RSV A occurred in the 2023/24 winter season, which is in line with neighbouring European countries. Lineage subtype distribution showed high concordance with circulating European strains. RSV A exhibited greater diversity (mean pairwise Hamming distance 0.015, SD 0.006) than RSV B (mean 0.006, 0.003), with all current strains falling within G protein duplication clades A.D.1, 2, 3 and 5 for RSV A, and B.D.E.1 and B.D.4.1.1 for RSV B.

CONCLUSION: By contributing 125 newly assembled RSV genome sequences, we have significantly increased the number of publicly available whole-genome sequences from Switzerland. Our study provides a genomic surveillance of RSV in Switzerland, analysing seasonal patterns, alternating subtype dominance, and consistency with broader European trends. A comprehensive understanding of the molecular epidemiology of RSV enables healthcare providers and public health authorities to monitor the effectiveness of current vaccines and monoclonal antibodies, associate lineages and serotypes with disease severity, implement timely interventions and detect emerging variants, ultimately reducing the burden of RSV-related illnesses.

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Introduction

Respiratory syncytial virus (RSV) is a single-stranded RNA virus that causes respiratory tract infections. RSV infection generally manifests with mild, cold-like symptoms but can cause severe complications in high-risk groups such as infants, older adults and immunocompromised patients [1]. Globally, RSV is the most common cause of lower respiratory tract infections in infants [2].

Prior to the COVID-19 pandemic, RSV activity in Switzerland followed a consistent seasonal pattern, typically commencing in October, peaking in December or January and subsiding by April. The number of hospitalisations exhibited a two-yearly regularity, where even-to-odd winter seasons showed significantly more hospitalisations than preceding ones [3]. However, from the first implementation of social distancing measures in late February 2020 to the post-pandemic winter seasons of 2022/23 and 2023/24, significant changes in RSV epidemiology have been observed [4–6]. These changes include shifts in the timing, intensity and age distribution of RSV infections, reflecting

the impact of public health measures and the subsequent relaxation of non-pharmaceutical interventions. While some have hypothesised that these shifts may be due to increased virulence of circulating RSV strains during the pandemic [7], current molecular epidemiological surveillance shows that post-pandemic circulation is dominated by several lineages with pre-pandemic roots, suggesting epidemiological rather than virological reasons [8].

With the recent introduction of monoclonal antibodies and prefusion F protein-based vaccines (RSVpreF) in 2023/24, such as nirsevimab (Beyfortus®) [9, 10] by AstraZeneca and Sanofi, Abrysvo® by Pfizer [11] and Arexvy® by GlaxoSmithKline [12], options for specific prevention of RSV infections have increased dramatically. These interventions raise important concerns about the molecular adaptation of RSV under selective pressure, possibly leading to variants that escape vaccine-induced immunity or develop resistance to monoclonal antibodies. The main focus of attention lies on the Fusion (F) protein, the primary antigen in current recombinant vaccines and a target for monoclonal antibodies [13]. Several studies so far have shown little to no elevated levels of polymorphisms in the vaccinees compared to the control group [14]. However, RSV is a rapidly evolving virus with a high mutation rate, and continuous monitoring is crucial to detect potential early signals of adaptation and escape variants that may arise as vaccines and monoclonal antibodies are rolled out more widely. A comprehensive understanding of the molecular epidemiology of RSV enables healthcare providers and public health authorities to monitor the effectiveness of current vaccines and monoclonal antibodies, assess the severity of RSV-related diseases, implement timely interventions and detect emerging variants, ultimately reducing the burden of RSV-related illnesses.

RSV is divided into two major antigenic subtypes, RSV A and RSV B, with the greatest divergence in the gene coding for the attachment glycoprotein G (G protein). The estimated common ancestor of the RSV A and RSV B circulated around 250 years ago [15]. A recent hierarchical classification by Goya et al. [16] defined 24 and 16 lineages within RSV A and RSV B, respectively, based on phylogeny and amino acid markers. This novel unified nomenclature is designed to be kept up-to-date through designation of new lineages with the aim of tracking epidemiologically relevant viral variants and comparing the circulation of the virus from season to season and across geographies.

In this study, we present data on RSV dynamics in Northwestern Switzerland during the pre-pandemic season 2019/20, the pandemic seasons 2020/21 and 2021/22, and the post-pandemic periods 2022/23 and 2023/24. Throughout this timeframe, we conducted nucleic acid testing and full-genome sequencing of RSV from symptomatic patients presenting with respiratory tract infections at our tertiary care hospital.

Methods

Patient cohorts and inclusion criteria

Patients included in our study presented with acute respiratory tract infections, as defined by at least 1 respiratory and 1 systemic symptom/sign such as clogged or runny nasal airways, sore throat, cough, fatigue, fever, headache, chills or myalgia [17]. This clinical diagnosis is a prerequisite for ordering broad molecular multiplex panel testing at our tertiary care hospital. The patients presented to the outpatient or emergency departments of University Hospital Basel or University Children's Hospital Basel between July 2019 and June 2024 and underwent RSV-specific nucleic acid testing. RSV-specific testing was performed using the Biofire FilmArray RPP (bioMérieux, Marcy-l'Étoile, France) and additionally the Xpert® Xpress-CoV-2/Flu/RSV-Plus system (Cepheid, CA, USA) [18, 19]. The sensitivity of the Biofire FilmArray RPP for detecting RSV ranges from 95% to 100%, while specificity exceeds 99%. The corresponding values for the Xpert® Xpress-CoV-2/Flu/RSV-Plus system are 95–98% sensitivity and >98% specificity [20, 21].

Amplicon-based next-generation sequencing approach

The 500bp-NAT (nucleic acid testing) primers were designed to match conserved sequences across all publicly available full-length RSV genome sequences from the NCBI nucleotide database (totalling 3010 as of 1 January 2022, with 2002 RSV A and 1008 RSV B). NAT primers were designed using the publicly available PRIMAL tool [22] that allows the design of highly multiplex primer pools. Additionally, 2000bp-NAT primers and 1000bp-RSV A and RSV B subgroup-specific NAT primers were employed from [23], and used in 2 separate primer pools. All NATs used the Iproof High Fidelity DNA Polymerase kit (BioRad, CA, USA) with 600 nM end concentration of the different primer pools (appendix figure S4, supplementary files 1 and 2). NATs had a reaction volume of 25 µl containing 5 µl of extracted DNA and were run on Veriti™ Thermal Cyclers (Applied Biosystems, MA, USA) using the thermal cycling protocol specified in the Iproof High Fidelity DNA Polymerase kit. Library preparation was done by pooling the six amplicons from the 2000bp, 1000bp and 500bp NAT reactions using the KAPA HyperPrep Kit (Roche, Rotkreuz, Switzerland) following the manufacturers' instructions. Next-generation sequencing (NGS) was performed on a MiniSeq platform (Illumina, CA, USA). Raw data, including FASTQ files, were subsequently processed and organised for downstream analysis.

Genome assembly

Raw sequencing reads were trimmed using Trim Galore v0.6.10 and mapped to reference genomes with BWA-MEM v0.7.18. Pileup analysis and consensus sequence construction were performed using custom scripts adapted from the Enterovirus D68 project [24, 25].

Preprocessing and sample selection

A total of 182 samples were initially selected for the analysis: 159 from outpatients and 23 from hospitalised patients with the best coverage (appendix figure S3). Preprocessing included masking positions with less than 90% main allele frequency. After applying a coverage threshold of at least 100× over 80% of the genome, 125 samples (96 RSV A, 29 RSV B) were retained for the phylogenetic analysis.

Phylogenetic analysis

Phylogenetic trees were constructed using the Nextstrain [26] CLI pipeline v8.5.3, which includes Augur v26.0.0 and Auspice v2.58.0. Consensus sequences were aligned with background data from the NCBI Virus database using Nextclade [27] v3.8.2, and trees were inferred using IQ-TREE [28] v2.3.6. Two trees were generated: one with no regional or temporal filters for global context and another focused on recent (last 3 years) European sequences for more detailed comparison. Diversity was calculated as follows: for each RSV subtype, aligned consensus sequences were taken; Hamming distance (the number of positions with different nucleotides for two aligned genomes) for each pair was calculated, excluding uncertain consensus positions and normalising to the length of the compared part. All the tools mentioned above are publicly available under GPL-3.0 or MIT licences.

Ethics statement

The study was conducted according to good laboratory practice and in accordance with the Declaration of Helsinki and national and institutional standards, and was approved by the ethics committee of Northwestern and Central Switzerland (EKNZ 2024-00813).

Results

Pre- and post-pandemic seasonal dynamics of RSV

Patients presenting with symptoms of respiratory tract infections at the outpatient or emergency departments of University Hospital Basel or University Children's Hospital Basel underwent RSV-specific nucleic acid testing between July 2019 and June 2024 (appendix table S2). RSV-positive samples with sufficient residual material further underwent full-genome sequencing, representing a convenience sample over the whole study period.

A total of 48,897 respiratory clinical specimens from 30,782 patients with respiratory tract infections – of whom 14,613 (47.5%) were female and 6436 (20.9%) paediatric patients <18 years – were submitted for routine RSV-specific nucleic acid testing between July 2019 and June 2024 at our tertiary care hospital in Northwestern Switzerland (table 1, appendix table S1). The median patient age was 62 years (range: 1 to 106 years).

First, we assessed the seasonality and peak activity of RSV during the study period. In 2019, prior to the COVID-19 pandemic, the number of RSV infections in our catchment area increased in October and subsided by February 2020, consistent with seasonal patterns observed before the pandemic [3]. The implementation of non-pharmaceutical interventions to reduce the spread of SARS-CoV-2 in 2020 and 2021, including travel restrictions, social distancing, mask mandates, and school and business closures, disrupted this regularity. Notably, during the 2020/21 season, RSV activity was minimal, with no significant epidemic observed. However, an atypical early surge occurred in 2021. Cases began to rise in May, peaking in July, and persisting until January 2022. Subsequent post-pandemic 2022/23 and 2023/24 seasons indicated a return to pre-pandemic seasonality, with activity commencing in late summer, peaking in November and declining by January (figure 1A).

The off-season activity in 2021 significantly impacted paediatric patients aged 1 to 5 years, who accounted for most RSV cases during that period. In subsequent seasons, we observed an increase in diagnosed RSV infections among adults and older patients aged ≥65 years (figure 1B). In the 2022/23 RSV season, RSV infections in infants and school-aged children peaked 1–2 months earlier than those in adults, which suggests that younger populations may have had higher susceptibility in the first post-pandemic season.

During the 2022/23 and 2023/24 seasons, adult populations experienced consecutive major RSV epidemics, leading to significantly increased RSV-related hospitalisations compared to the pandemic 2019/20 and 2020/21 seasons (appendix figure S1). The elevated case numbers in post-pandemic seasons relative to pre-pandemic seasons can be attributed, in part, to increased testing rates, as test positivity does not differ as dramatically between pre- and post-pandemic seasons. Hospitalisation rates for paediatric patients were not available for this study, but recent data suggest that immunologically naive children or those with limited prior exposure to RSV during the COVID-19 pandemic experienced more severe RSV-related illness and faced a higher risk of hospitalisation compared to pre-pandemic years [5, 29, 30].

Seasonal distribution of RSV subtypes

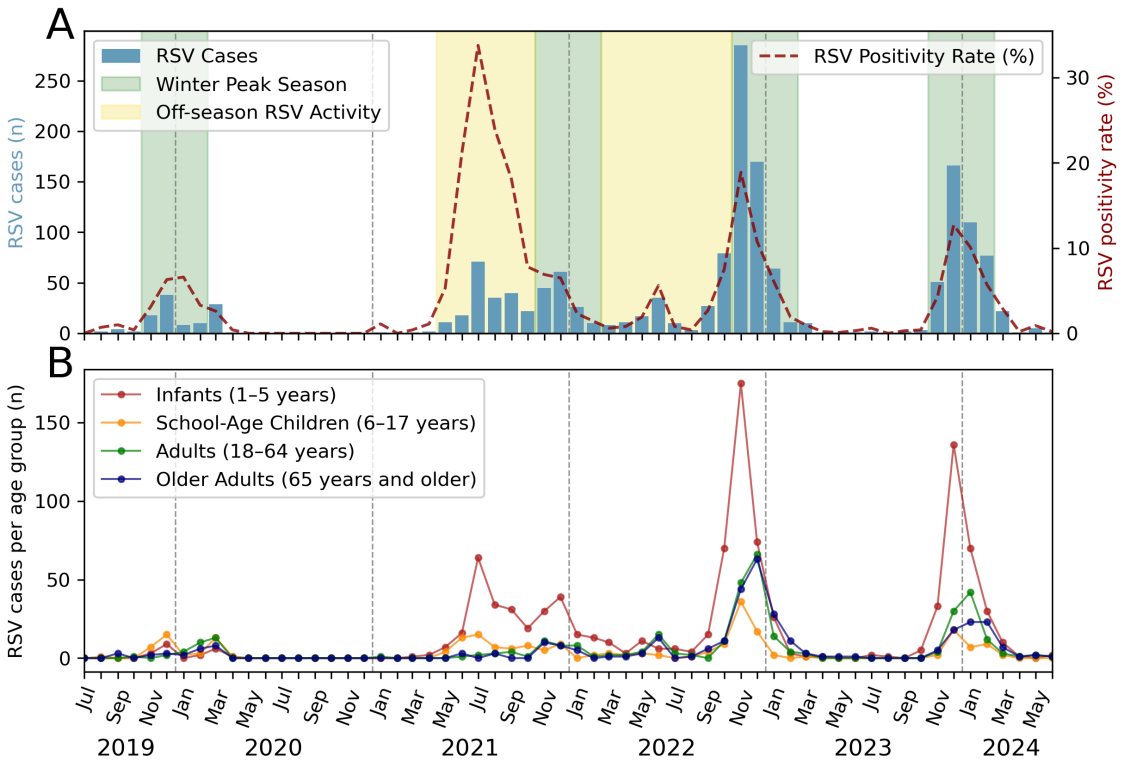
Respiratory viruses generally show a similar distribution of genotypes in geographically well-connected regions, but during the pandemic period with travel restrictions and variable approaches to social distancing and non-pharmaceutical interventions, resolving the local diversity of viruses is of particular interest. Furthermore, addressing the hypotheses that circulating RSV strains after the pandemic may have exhibited increased virulence (appendix table ~~S3),~~ potentially contributing to higher transmission rates, increased case numbers and related hospitalisations, requires whole-genome sequencing of a sufficiently large number of viral samples to detect or rule out strong associations between viral genotypes and presentation.

While only a few samples were available for whole-genome sequencing prior to 2022, we generated 20 full genomes from the 2022/23 season and 100 for the 2023/24

Table 1:
Distribution of tested and sequenced samples.

Demographic	2019		2020		2021		2022		2023		2024		Total	
	<18	≥18	<18	≥18	<18	≥18	<18	≥18	<18	≥18	<18	≥18	<18	≥18
RSV-positive samples (n)	43	21	28	43	310	55	481	310	231	123	131	118	1224	670
RSV-positive patients (n)	43	21	18	30	252	55	433	248	214	98	117	99	1077	551
RSV sequences (n)				2		7		23	69	41	16	24	85	97
RSV sequences, passed QC (n)				1		3		13	56	30	6	16	62	63

Figure 1: Respiratory syncytial virus (RSV) seasonality, peak activity and demographic distribution. (A) RSV detections during the pandemic seasons (2019/20, 2020/21, 2021/22) and the post-pandemic period (2022/23, 2023/24). The green background bar represents the typical RSV winter season, running from November to February, and the yellow background bar indicates off-season RSV activity. The number of positive cases per month is indicated by blue bars and the positive rate is shown as a dashed red line (right axis). (B) Age distribution of RSV cases by month for the specified period.

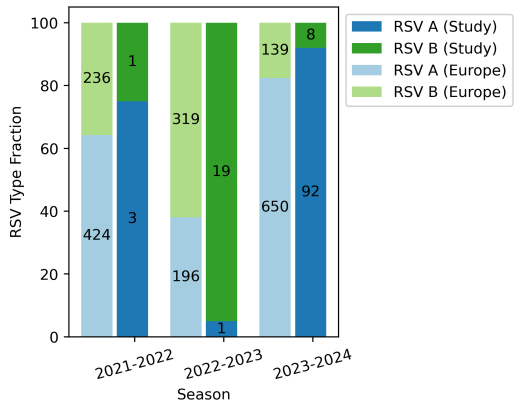


season. As shown in figure 2, RSV B dominated during the 2022/23 season (19 of 20 samples), while RSV A was the main subtype in the 2023/24 season (92 of 100 samples).

Phylogenetic analysis of RSV subtypes

All currently circulating RSV strains belong to clades with duplications in the G protein (figures 3A and 4A), which occurred independently in RSV A and RSV B [31, 32] in 2009 and 1996, respectively, according to the reconstructed phylogenies. These clades are named A.D and B.D in the new lineage nomenclature [16] and have been divided into several sublineages.

Figure 2: Annual distribution of RSV A and RSV B subtypes in Switzerland and Europe over the 2021/22, 2022/23 and 2023/24 seasons. Numbers inside the bars display actual numbers of respiratory syncytial virus (RSV) subtype cases, while heights correspond to fractions. Stacked bars to the left show same-season RSV subtype distributions in European samples available in the NCBI virus database.



RSV A: Switzerland vs Europe

Phylogenetic analysis of RSV A sequences revealed a high degree of correspondence between Switzerland and the broader European context (figures 3B–C). Sequence diversity levels are higher for RSV A, with mean pairwise Hamming distance of 0.015 and SD 0.006, compared to the mean of 0.006 and SD 0.003 for RSV B (appendix figure S2). RSV A samples were found in three main lineages – A.D.1, A.D.3 and A.D.5 – and, to a lesser extent, in A.D.2. These lineages have been observed across Europe and have a common ancestor prior to 2015. Therefore, multiple lineages of RSV A have persisted from the pre-pandemic period and there is no indication that a particular lineage with distinct properties has emerged that might be associated with a different presentation. Overall, this diverse RSV A population with high correspondence to European dynamics suggests that RSV A was introduced into Switzerland numerous times each season and that there is frequent exchange with neighbouring countries.

RSV B: Switzerland vs Europe

The RSV B phylogeny also showed high correspondence with other European countries. Current circulating strains of RSV B mostly fall within B.D.E.1 and B.D.4.1.1 clades,

which is also the case for our data (figures 4B-C, appendix table S4): 26 samples belong to B.D.E.1 and 3 samples to its parent clade, B.D.4.1.1. The lineage B.D.E.1 emerged shortly before the pandemic and has expanded globally over the past 5 years. This recent expansion explains the lower Hamming distance levels compared to RSV A and the different clade distribution. Apart from these differences between subtypes, RSV B samples also display multiple introduction events with many lineages persisting across many seasons.

Figure 3: RSV A phylogeny. (A) Global phylogenetic tree of RSV A sequences. (B) RSV A phylogenetic tree focusing on the last two years of European data. Bold leaves indicate sequences generated in this study. (C) Geographic distribution of RSV A clades in Europe over the last two seasons.

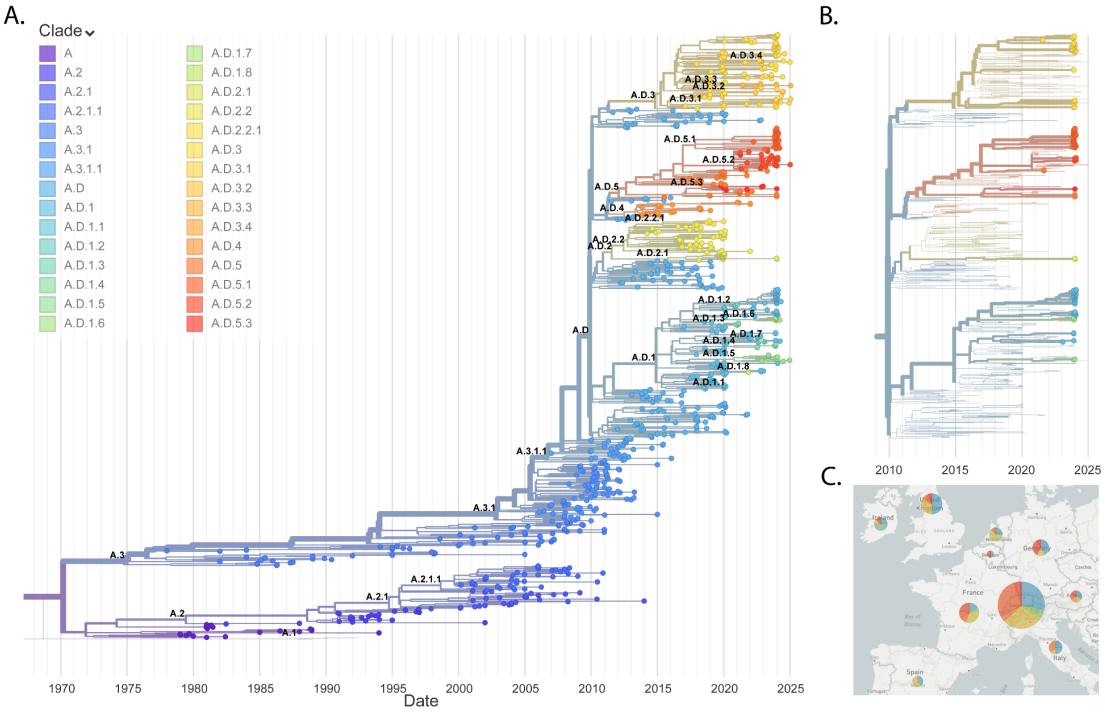
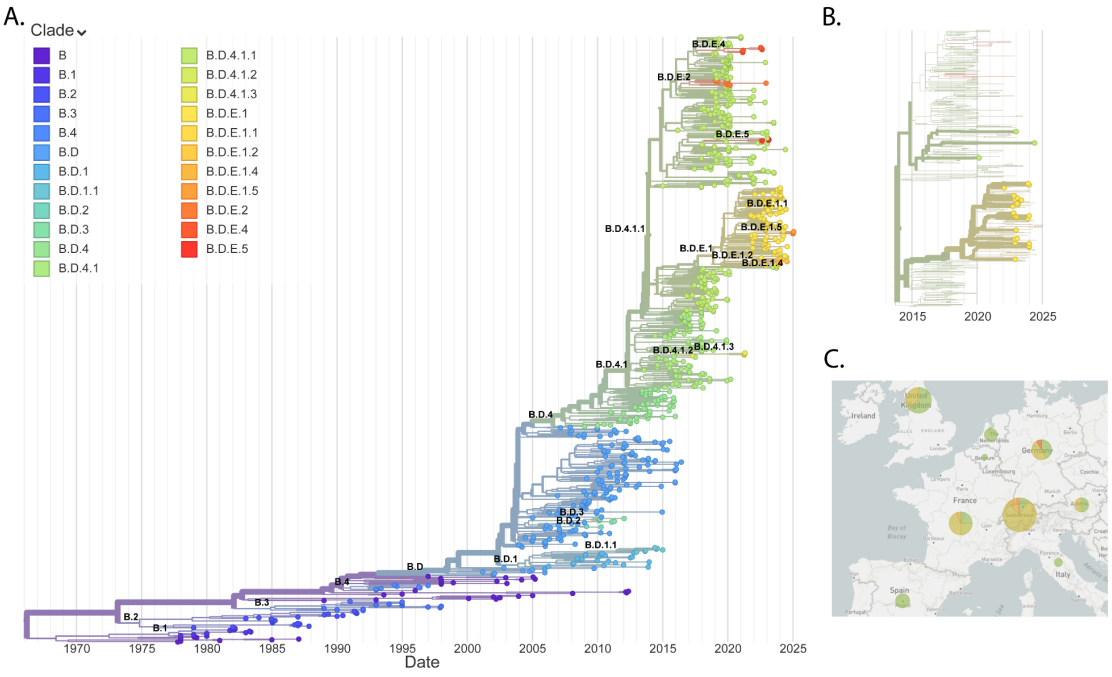


Figure 4: RSV B phylogeny. (A) Global phylogenetic tree of RSV B sequences. (B) RSV B phylogenetic tree focusing on the last two years of European data. Bold leaves indicate sequences generated in this study. (C) Geographic distribution of RSV B clades in Europe during the last two seasons.



Discussion

The COVID-19 pandemic has significantly disrupted the typical seasonal patterns of RSV transmission and activity [33]. Public health measures implemented to curb the spread of SARS-CoV-2, such as social distancing, mask-wearing and lockdowns, led to a temporary decline in RSV cases [34]. Here, we reported on the patterns of RSV infections and hospitalisations during this period in Northwestern Switzerland. As these measures were relaxed, a notable resurgence of RSV activity was observed. For example, an unusual outbreak occurred in the summer of 2021, deviating from the typical winter peak. This was followed by a strong resurgence of RSV cases and related hospitalisations in the following 2022/23 and 2023/24 seasons after all COVID-19 restrictions were lifted. The observed patterns of RSV infections generally mirror those observed elsewhere in Switzerland [5, 6], but are notably different from those of the neighbouring countries Germany [35–37], Italy [38–40] and Austria [41], where no summer wave was observed in 2021. The USA, on the other hand, did observe significant RSV activity between April 2021 and February 2022 [42]. Considering the varied resurgence patterns of RSV across Europe, one might anticipate that the dominant viral type (RSV A vs B) differs between countries, or that different sublineages of RSV A or B circulated in different regions. To investigate this hypothesis, we performed whole-genome sequencing on respiratory swabs, generating a total of 125 RSV genomes. Overall, the dominance patterns of RSV A and B were consistent with those of neighbouring countries. However, Europe as a whole demonstrated a moderately more even distribution of both viral types, which might be due to the much larger and diverse demographic.

Aggregated data from Europe for these two seasons reveal the presence of the same predominant subtypes. However, the dominance is less pronounced: RSV B accounted for 62% of the samples in the 2022/23 season (319 of 515) while RSV A comprised 82% of the samples in the 2023/24 season (650 of 789). Data from the neighbouring countries Austria and Germany indicate a similarly pronounced alternation in subtype dominance, exhibiting a 90% difference between these seasons [37, 41], akin to the trends observed in Switzerland. An analysis of wastewater samples in Switzerland found the same subtype distribution for post-pandemic seasons [43]. In contrast, the USA exhibits the opposite pattern in seasonal subtype dynamics: RSV B was predominant in the 2021/22 and 2023/24 seasons, while RSV A prevailed during the 2022/23 season [42, 44]. This disparity underscores the necessity for global surveillance of RSV.

Concordance was similarly observed at the lineage level. For RSV A, these sequenced strains reflect lineages that were prevalent in Europe during the same period. Most of these lineages have been circulating in parallel since 2010, predating the pandemic, which implies that no specific lineage was responsible for the resurgence of cases. Instead, observed differences in disease presentation and incidence are likely attributable to epidemiological or immunological factors arising from non-pharmaceutical interventions and reduced exposure to viral pathogens during periods of social distancing. Conversely, since the pandemic's onset, RSV B diversity has primarily been driven by lineage B.D.E.1 and its descendants, which likely arose more recently in 2019 [45].

Consequently, RSV B sequences exhibit greater similarity to one another compared to RSV A sequences. Notably, the diversity that we observed in Northwestern Switzerland aligns closely with the circulating strains in neighbouring European countries.

These findings indicate a sufficiently rapid exchange of RSV among European countries, facilitating alignment of the circulation patterns, even amid restricted travel and movement. However, on a broader geographic scale, these patterns exhibit notable differences. For instance, in the United States, different dominance patterns have been observed for RSV A and B, reflecting a divergence in the epidemiological behaviour of the virus compared to Europe [42].

Monitoring the circulating strains of RSV is crucial, as RSV subtypes A and B co-circulate globally, with shifts in predominance observed over time. Research has demonstrated that these subtypes may present different clinical manifestations and disease outcomes. In paediatric populations, it has been shown that RSV A infections tend to result in more severe clinical presentations and poorer patient outcomes compared to RSV B [46–48]. Although fewer studies have focused on adult populations, existing evidence suggests that RSV A is associated with increased virulence, as indicated by clinical severity scores [49]. These findings align with our observations, as the RSV A-dominant 2023/24 season coincided with an increase in hospitalisation rates, although causality cannot be established within the scope of our study. As discussed above, the genetic diversity of RSV A observed during the post-pandemic resurgence does not imply that lineages that persisted through the pandemic possessed unique characteristics.

This highlights the importance of accurately identifying circulating RSV strains. Understanding which strains are prevalent allows healthcare providers and public health authorities to better anticipate potential increases in transmission rates, case numbers and disease severity, facilitating more effective allocation of healthcare resources and implementation of targeted interventions.

With the introduction of RSV vaccines for adults in Switzerland, such as Arexvy and Abryso, alongside monoclonal antibodies for children, including Beyfortus, monitoring the molecular epidemiology of RSV and tracking escape or resistance mutations is becoming increasingly important. While little resistance has been observed in clinical studies to date, the widespread deployment of these preventive measures may exert additional pressure on the virus to evolve [50]. Early identification of such strains is essential for adapting diagnostic tools, refining prevention strategies and enhancing vaccine strategies to maintain their effectiveness against RSV infections.

This study has several limitations that should be acknowledged. The limited catchment area restricts the generalisability of the findings to broader populations, potentially overlooking regional variations in RSV circulation. The use of convenience sampling for full genome analysis may introduce selection bias, as samples are not randomly chosen and may not accurately represent the entire circulating

viral diversity. Additionally, the coverage bias inherent in next-generation sequencing (NGS) results, which excludes low-coverage samples from analysis, could influence the observed lineage distribution by underrepresenting certain variants that are present at lower abundance. Relying solely on consensus sequences further limits the detection of intra-host viral diversity and minority variants, potentially overlooking minor lineages or mutations that could impact viral evolution and epidemiology. Lastly, detailed clinical information, including patient outcomes, precise anatomical location of infection (upper and lower respiratory tract infections) and disease severity, was not available in the scope of this study. Therefore correlations between viral genotypes and these factors could not be assessed, limiting epidemiological conclusions to subtype occurrence analysis only. These methodological constraints may collectively influence the study's conclusions regarding RSV lineage dynamics and mask the full spectrum of viral diversity within the studied population.

In summary, our analysis of RSV epidemiology of recent seasons in Switzerland outlines the dynamics of case numbers and subtype prevalence and highlights the importance of sustained surveillance. Ongoing monitoring is critical to inform public health policies, guide vaccination strategies and detect the emergence of variants with potential clinical and epidemiological significance.

Data sharing statement

The consensus genomes generated in this study have been submitted to the Pathoplexus database under sequence sets PP_SS_201.2 [51] and PP_SS_202.2 [52] and to NCBI Datasets Virus Data under Bioprojects PRJEB88486 and PRJEB88624, respectively.

Code availability: The custom algorithms and scripts developed for this study are openly accessible and can be found in our GitHub repository at: https://github.com/neherlab/rsv_epidemiology_2025

We encourage researchers and practitioners to explore and use this resource for further advancements in the field.

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Author contributions: RG, RAN and KL contributed to the conceptualisation and study design. Data collection and clinical contributions were provided by UH, NK and STS. Methodology and laboratory analyses were conducted by RG and KL. Data analysis and interpretation were performed by AK, RG, RAN and KL. The original draft of the manuscript was written by AK and RAN. All authors reviewed and approved the final manuscript.

Potential competing interests

NK has served on safety and advisory boards for Idorsia and Pulmicide and advisory boards for Gilead, MSD, Pfizer and Takeda. – RAN has received consulting fees from Moderna TX and BioNTech. – UH is a member of the Meta Data Safety Monitoring Board of CEPI.

References

1. Kaler J, Hussain A, Patel K, Hernandez T, Ray S. Respiratory syncytial virus: A comprehensive review of transmission, pathophysiology, and manifestation. Cureus. 2023 Mar;15(3):e36342. http://dx.doi.org/10.7759/cureus.36342.
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012 Dec;380(9859):2095–128. http://dx.doi.org/10.1016/S0140-6736(12)61728-0.
3. Stucki M, Lenzin G, Agyeman PK, Posfay-Barbe KM, Ritz N, Trück J, et al. Inpatient burden of respiratory syncytial virus (RSV) in Switzerland, 2003 to 2021: an analysis of administrative data. Euro Surveill. 2024 Sep;29(39):2400119. http://dx.doi.org/10.2807/1560-7917.ES.2024.29.39.2400119.
4. Meslé MM, Sinnathamby M, Mook P, Pebody R, Lakhani A, Zambon M, et al.; WHO European Region Respiratory Network Group. Seasonal and inter-seasonal RSV activity in the European Region during the COVID-19 pandemic from autumn 2020 to summer 2022. Influenza Other Respir Viruses. 2023 Nov;17(11):e13219. http://dx.doi.org/10.1111/irv.13219.
5. Fischli K, Schöbi N, Duppenthaler A, Casaulta C, Riedel T, Kopp MV, et al. Postpandemic fluctuations of regional respiratory syncytial virus hospitalization epidemiology: potential impact on an immunization program in Switzerland. Eur J Pediatr. 2024 Dec;183(12):5149–61. http://dx.doi.org/10.1007/s00431-024-05785-z.
6. Sauteur PM, Plebani M, Trück J, Wagner N, Agyeman PK. Ongoing disruption of RSV epidemiology in children in Switzerland. Lancet Reg Heal; 2024. p. 45.
7. Rao S, Armistead I, Messacar K, Alden NB, Schmoll E, Austin E, et al. Shifting epidemiology and severity of respiratory syncytial virus in children during the COVID-19 pandemic. JAMA Pediatr. 2023 Jul;177(7):730–2. http://dx.doi.org/10.1001/jamapediatrics.2023.1088.
8. Abu-Raya B, Viñeta Paramo M, Reicherz F, Lavoie PM. Why has the epidemiology of RSV changed during the COVID-19 pandemic? EClinicalMedicine. 2023 Jul;61:102089. http://dx.doi.org/10.1016/j.eclinm.2023.102089.
9. Hammitt LL, Dagan R, Yuan Y, Baca Cots M, Bosheva M, Madhi SA, et al.; MELODY Study Group. Nirsevimab for prevention of RSV in healthy late-preterm and term infants. N Engl J Med. 2022 Mar;386(9):837–46. http://dx.doi.org/10.1056/NEJMoa2110275.
10. Griffin MP, Yuan Y, Takas T, Domachowske JB, Madhi SA, Manzoni P, et al.; Nirsevimab Study Group. Single-dose nirsevimab for prevention of RSV in preterm infants. N Engl J Med. 2020 Jul;383(5):415–25. http://dx.doi.org/10.1056/NEJMoa1913556.
11. Simões EA, Pahud BA, Madhi SA, Kampmann B, Shittu E, Radley D, et al.; MATISSE (Maternal Immunization Study for Safety and Efficacy) Clinical Trial Group. Efficacy, Safety, and Immunogenicity of the MATISSE (Maternal Immunization Study for Safety and Efficacy) Maternal Respiratory Syncytial Virus Prefusion F Protein Vaccine Trial. Obstet Gynecol. 2025 Feb;145(2):157–67. http://dx.doi.org/10.1097/AOG.0000000000005816.
12. Shirley M. RSVPreF3 OA respiratory syncytial virus vaccine in older adults: a profile of its use. Drugs Ther Perspect. 2025;41(1):1–8. http://dx.doi.org/10.1007/s40267-024-01125-1.
13. Papi A, Ison MG, Langley JM, Lee DG, Leroux-Roels I, Martinon-Torres F, et al.; AReSVi-006 Study Group. Respiratory syncytial virus prefusion F protein vaccine in older adults. N Engl J Med. 2023 Feb;388(7):595–608. http://dx.doi.org/10.1056/NEJMoa2209604.
14. Fourati S, Reslan A, Bourret J, Casalegno JS, Rahou Y, Chollet L, et al. Genotypic and phenotypic characterisation of respiratory syncytial virus after nirsevimab breakthrough infections: a large, multicentre, observational, real-world study. Lancet Infect Dis. 2024.
15. Saito M, Tsukagoshi H, Sada M, Sunagawa S, Shirai T, Okayama K, et al. Detailed evolutionary analyses of the F gene in the respiratory syncytial virus subgroup A. Viruses. 2021 Dec;13(12):2525. http://dx.doi.org/10.3390/v13122525.
16. Goya S, Ruis C, Neher RA, Meijer A, Aziz A, Hinrichs AS, et al. Standardized phylogenetic classification of human respiratory syncytial virus below the subgroup level. Emerg Infect Dis. 2024 Aug;30(8):1631–41. http://dx.doi.org/10.3201/eid3008.240209.
17. Ison MG, Hirsch HH. Community-acquired respiratory viruses in transplant patients: diversity, impact, unmet clinical needs. Clin Microbiol Rev. 2019 Sep;32(4):10–1128. http://dx.doi.org/10.1128/CMR.00042-19.
18. Goldenberger D, Leuzinger K, Sogaard KK, Gosert R, Roloff T, Naegele K, et al. Brief validation of the novel GeneXpert Xpress SARS-CoV-2 PCR assay. J Virol Methods. 2020 Oct;284:113925. http://dx.doi.org/10.1016/j.jviromet.2020.113925.
19. Leuzinger K, Roloff T, Gosert R, Sogaard K, Naegele K, Rentsch K, et al. Epidemiology of severe acute respiratory syndrome coronavirus 2 emergence amidst community-acquired respiratory viruses. J Infect Dis. 2020 Sep;222(8):1270–9. http://dx.doi.org/10.1093/infdis/jiaa464.
20. Leung EC man, Chow VC ying, Lee MK ping, Tang KP san, Li DK cheung, Lai RW man. Evaluation of the Xpert Xpress SARS-CoV-2/Flu/

RSV Assay for Simultaneous Detection of SARS-CoV-2, Influenza A and B Viruses, and Respiratory Syncytial Virus in Nasopharyngeal Specimens. Hayden R, editor. J Clin Microbiol. 2021 Mar 19;59(4):e02965-20.
21. Mostafa HH, Carroll KC, Hicken R, Berry GJ, Manji R, Smith E, et al. Multicenter Evaluation of the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV Test. McAdam AJ, editor. J Clin Microbiol. 2021 Feb 18;59(3):e02955-20.
22. Quick J, Grubaugh ND, Pullan ST, Claro IM, Smith AD, Gangavarapu K, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc. 2017 Jun;12(6):1261–76. http://dx.doi.org/10.1038/nprot.2017.066.
23. Wang L, Ng TF, Castro CJ, Marine RL, Magaña LC, Esona M, et al. Next-generation sequencing of human respiratory syncytial virus subgroups A and B genomes. J Virol Methods. 2022 Jan;299:114335. http://dx.doi.org/10.1016/j.jviromet.2021.114335.
24. Dyrdak R, Mastafa M, Hodcroft EB, Neher RA, Albert J. Intra- and interpatient evolution of enterovirus D68 analyzed by whole-genome deep sequencing. Virus Evol. 2019 Apr;5(1):vez007. http://dx.doi.org/10.1093/ve/vez007.
25. Hodcroft EB, Dyrdak R, Andrés C, Egli A, Reist J, García Martínez De Artola D, et al. Evolution, geographic spreading, and demographic distribution of Enterovirus D68. Elde NC, editor. PLOS Pathog. 2022 May 31;18(5):e1010515. http://dx.doi.org/10.1371/journal.ppat.1010515.
26. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018 Dec;34(23):4121–3. http://dx.doi.org/10.1093/bioinformatics/bty407.
27. Aksamentov I, Roemer C, Hodcroft EB, Neher RA. Nextclade: clade assignment, mutation calling and quality control for viral genomes. J Open Source Softw. 2021;6(67):3773. http://dx.doi.org/10.21105/joss.03773.
28. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015 Jan;32(1):268–74. http://dx.doi.org/10.1093/molbev/msu300.
29. Garcia-Maurino C, Brenes-Chacón H, Halabi KC, Sánchez PJ, Ramilo O, Mejias A. Trends in age and disease severity in children hospitalized with RSV infection before and during the COVID-19 pandemic. JAMA Pediatr. 2024 Feb;178(2):195–7. http://dx.doi.org/10.1001/jamapediatrics.2023.5431.
30. Bourdeau M, Vadlamudi NK, Bastien N, Embree J, Halperin SA, Jadavji T, et al.; Canadian Immunization Monitoring Program Active (IMPACT) Investigators. Pediatric RSV-associated hospitalizations before and during the COVID-19 pandemic. JAMA Netw Open. 2023 Oct;6(10):e2336863–2336863. http://dx.doi.org/10.1001/jamanetworkopen.2023.36863.
31. Eshaghi A, Duvvuri VR, Lai R, Nadarajah JT, Li A, Patel SN, et al. Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. PLoS One. 2012;7(3):e32807. http://dx.doi.org/10.1371/journal.pone.0032807.
32. Trento A, Galiano M, Videla C, Carballal G, García-Barreno B, Melero JA, et al. Major changes in the G protein of human respiratory syncytial virus isolates introduced by a duplication of 60 nucleotides. J Gen Virol. 2003 Nov;84(Pt 11):3115–20. http://dx.doi.org/10.1099/vir.0.19357-0.
33. Stein RT, Zar HJ. RSV through the COVID-19 pandemic: Burden, shifting epidemiology, and implications for the future. Pediatr Pulmonol. 2023 Jun;58(6):1631–9. http://dx.doi.org/10.1002/ppul.26370.
34. Heemskerk S, Baliatsas C, Stelma F, Nair H, Paget J, Spreeuwenberg P. Assessing the Impact of Non-Pharmaceutical Interventions During the COVID-19 Pandemic on RSV Seasonality in Europe. Influenza Other Respir Viruses. 2025 Jan;19(1):e70066. http://dx.doi.org/10.1111/irv.70066.
35. Scholz S, Dobrindt K, Tufts J, Adams S, Ghaswalla P, Ultsch B, et al. The Burden of Respiratory Syncytial Virus (RSV) in Germany: A Comprehensive Data Analysis Suggests Underdetection of Hospitalisations and Deaths in Adults 60 Years and Older. Infect Dis Ther. 2024 Aug;13(8):1759–70. http://dx.doi.org/10.1007/s40121-024-01006-0.
36. Kiefer A, Pemmerl S, Kabesch M, Ambrosch A. Comparative analysis of RSV-related hospitalisations in children and adults over a 7 year-period before, during and after the COVID-19 pandemic. J Clin Virol. 2023 Sep;166:105530. http://dx.doi.org/10.1016/j.jcv.2023.105530.
37. Hönemann M, Maier M, Frille A, Thiem S, Bergs S, Williams TC, et al. Respiratory Syncytial Virus in Adult Patients at a Tertiary Care Hospital in Germany: Clinical Features and Molecular Epidemiology of the Fusion Protein in the Severe Respiratory Season of 2022/2023. Viruses. 2024 Jun;16(6):943. http://dx.doi.org/10.3390/v16060943.
38. Lastrucci V, Pacifici M, Puglia M, Alderotti G, Berti E, Del Riccio M, et al. Seasonality and severity of respiratory syncytial virus during the COVID-19 pandemic: a dynamic cohort study. Int J Infect Dis. 2024 Nov;148:107231. http://dx.doi.org/10.1016/j.ijid.2024.107231.
39. Pierangeli A, Nenna R, Fracella M, Scagnolari C, Oliveto G, Sorrentino L, et al. Genetic diversity and its impact on disease severity in respiratory syncytial virus subtype-A and -B bronchiolitis before and after pandemic restrictions in Rome. J Infect. 2023 Oct;87(4):305–14. http://dx.doi.org/10.1016/j.jinf.2023.07.008.
40. Cutrera R, Ciofi Degli Atti ML, Dotta A, D'Amore C, Ravà L, Perno CF, et al. Epidemiology of respiratory syncytial virus in a large pediatric hospital in Central Italy and development of a forecasting model to predict the seasonal peak. Ital J Pediatr. 2024 Apr;50(1):65. http://dx.doi.org/10.1186/s13052-024-01624-x.
41. Redlberger-Fritz M, Springer DN, Aberle SW, Camp JV, Aberle JH. Respiratory syncytial virus surge in 2022 caused by lineages already present before the COVID-19 pandemic. J Med Virol. 2023 Jun;95(6):e28830. http://dx.doi.org/10.1002/jmv.28830.
42. Rios-Guzman E, Simons LM, Dean TJ, Agnes F, Pawlowski A, Alisoltanidehkordi A, et al. Deviations in RSV epidemiological patterns and population structures in the United States following the COVID-19 pandemic. Nat Commun. 2024 Apr;15(1):3374. http://dx.doi.org/10.1038/s41467-024-47757-9.
43. de Korne-Elenbaas J, Rimaite A, Topolsky I, Dreifuss D, Bürki C, Fuhrmann L, et al. Wastewater-based sequencing of Respiratory Syncytial Virus enables tracking of lineages and identifying mutations at antigenic sites. medRxiv [Preprint]. 2025 medRxiv [cited 2025 Mar 7]. p. 2025.02.28.25321637. Available from: https://www.medrxiv.org/content/10.1101/2025.02.28.25321637v1 http://dx.doi.org/10.1101/2025.02.28.25321637.
44. Yunker M, Fall A, Norton JM, Abdullah O, Villafuerte DA, Pekosz A, et al. Genomic Evolution and Surveillance of Respiratory Syncytial Virus during the 2023-2024 Season. Viruses. 2024 Jul;16(7):1122. http://dx.doi.org/10.3390/v16071122.
45. de Jesus-Cornejo J, Hechanova-Cruz RA, Sornillo JB, Okamoto M, Oshitani H; Viral Etiology Working Group. Prolonged RSV circulation in 2022 to 2023 associated with the emergence of a novel RSV-B clade in Biliran, Philippines. J Infect. 2025 Apr;90(4):106467. http://dx.doi.org/10.1016/j.jinf.2025.106467.
46. Jafri HS, Wu X, Makari D, Henrickson KJ. Distribution of respiratory syncytial virus subtypes A and B among infants presenting to the emergency department with lower respiratory tract infection or apnea. Pediatr Infect Dis J. 2013 Apr;32(4):335–40. http://dx.doi.org/10.1097/INF.0b013e318282603a.
47. Midulla F, Nenna R, Scagnolari C, Petrarca L, Frassanito A, Viscido A, et al. How respiratory syncytial virus genotypes influence the clinical course in infants hospitalized for bronchiolitis. J Infect Dis. 2019 Jan;219(4):526–34. http://dx.doi.org/10.1093/infdis/jiy496.
48. Jung JA, Wi PH, Kim H. Kim H sol. Comparisons of clinical characteristics between Respiratory Syncytial Virus A and B infection. J Allergy Clin Immunol. 2020;145(2):AB131. http://dx.doi.org/10.1016/j.jaci.2019.12.520.
49. Vos LM, Oosterheert JJ, Kuil SD, Viveen M, Bont LJ, Hoepelman AI, et al. High epidemic burden of RSV disease coinciding with genetic alterations causing amino acid substitutions in the RSV G-protein during the 2016/2017 season in The Netherlands. J Clin Virol. 2019 Mar;112:20–6. http://dx.doi.org/10.1016/j.jcv.2019.01.007.
50. Irwin KK, Renzette N, Kowalik TF, Jensen JD. Antiviral drug resistance as an adaptive process. Virus Evol. 2016 Jun;2(1):vew014. http://dx.doi.org/10.1093/ve/vew014.
51. Gosert R, Leuzinger K, Kuznetsov A, Neher RA. Respiratory syncytial virus A amplicon sequencing dataset, Basel, Switzerland, 2021-2024 [dataset]. 2025 Jun 16 [cited 2025 Sep 23]. Pathoplexus. Available from: http://dx.doi.org/10.62599/PP_SS_201.2.
52. Gosert R, Leuzinger K, Kuznetsov A, Neher RA. Respiratory syncytial virus B amplicon sequencing dataset, Basel, Switzerland, 2020-2024 [dataset]. 2025 Jun 16 [cited 2025 Sep 23]. Pathoplexus. Available from: http://dx.doi.org/10.62599/PP_SS_202.2.

Appendix

The supplementary files are available for download as separate files at https://doi.org/10.57187/s.4600.

Molecular Epidemiology of Respiratory Syncytial Virus in Switzerland 2019-2024 from Nucleic Acid Testing and Whole Genome Sequencing

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Supplementary Figures

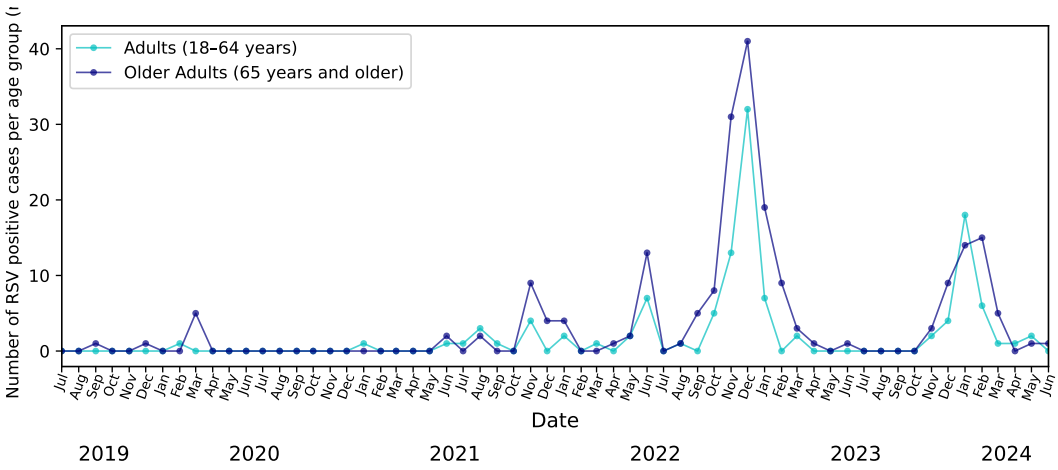

Figure S1: **RSV-related hospitalizations in adult populations across the 2019-24 seasons.** RSV-related hospitalizations by month for the pandemic seasons (2019–20, 2020–21, and 2021–22) and the post-pandemic period (2022–23 and 2023–24) in University Hospital Basel. Seasonal distribution is in line with RSV detections data from Figure 1, showing 2021 and 2022 off-season RSV activity and return to pre-pandemic seasonality in 2023.

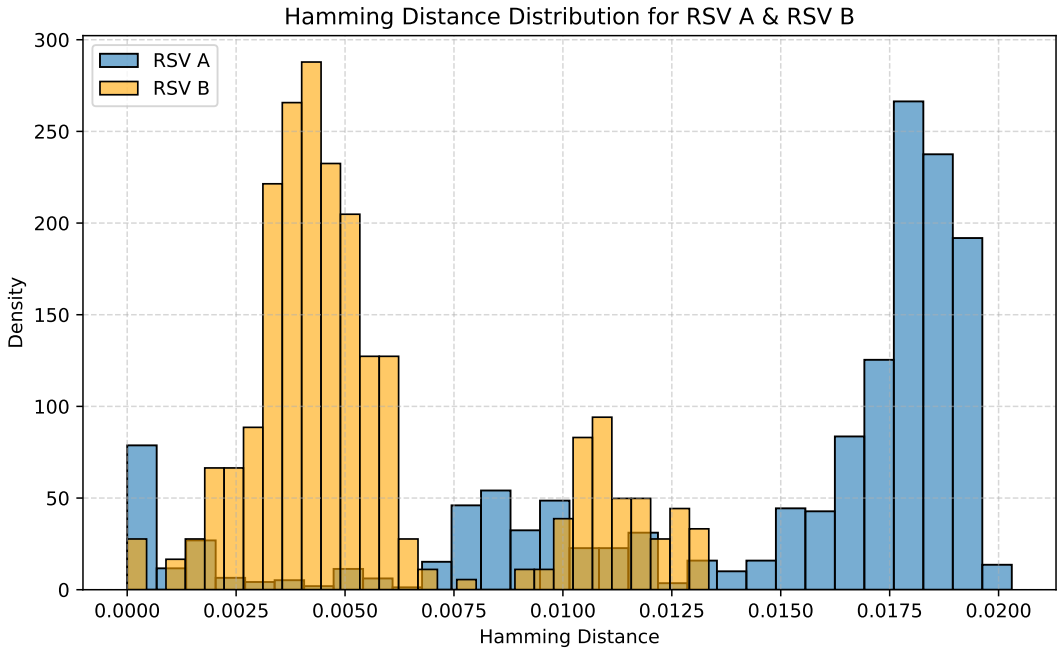

Figure S2: **Pairwise Hamming distance within RSV A and B samples.** Histogram of Hamming distances shows an objective measure of similarities among consensus genomes within each subtype: n=96 for RSV A and n=29 for RSV B. RSV A samples show a broader and multimodal distribution (mean=0.015, SD=0.006), consistent with the presence of three evenly represented clades (A.D.1, A.D.3, A.D.5), with inter-clade sample pairs contributing to the rightmost blue peak. RSV B samples exhibit a narrower distribution (mean=0.006, SD=0.003), with 26 out of 29 sequences belonging to the B.D.E.1 clade, forming the dominant leftmost golden peak.

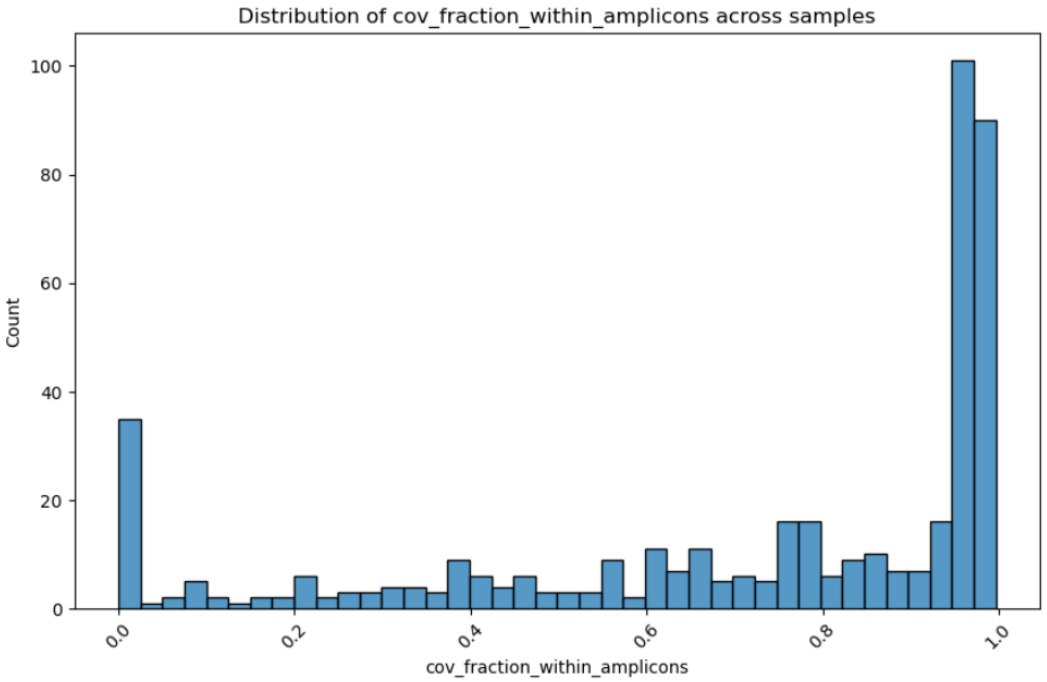

Figure S3: **Coverage distribution for sequenced RSV samples.** Consensus genomes were reconstructed for 182 sequenced samples. Based on the ≥100x coverage distribution histogram, 80% threshold was applied, retaining a total of 125 samples for the downstream phylogenetic analysis.

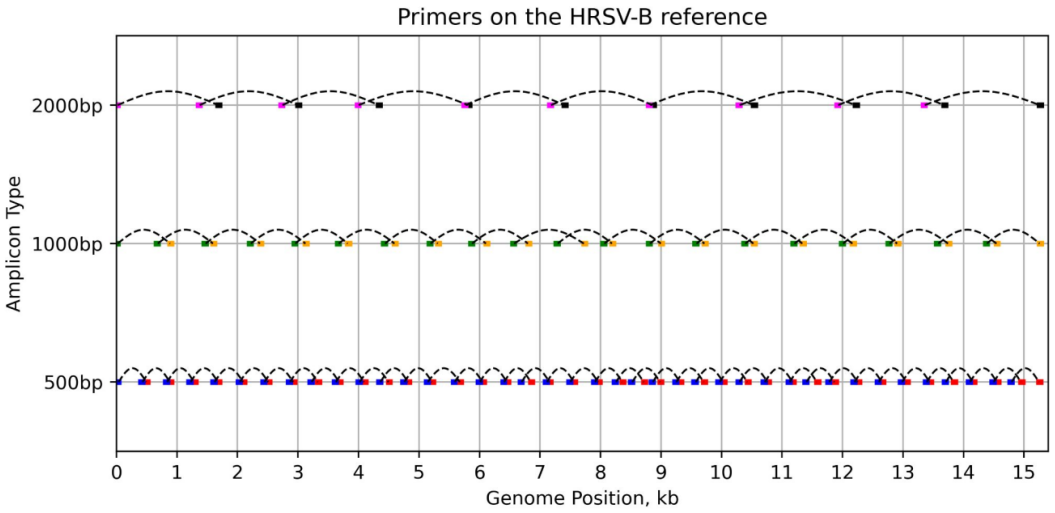

Figure S4: **Primer scheme for RSV B genome amplification.** The applied tiling amplicon scheme includes 2000bp, 1000bp, and 500bp NAT reactions, each split into even and odd pools (6 total reactions). Amplicons were pooled prior to library preparation. The X axis shows genomic positions on the RSV B reference genome, and the Y axis indicates amplicon types. Colors differentiate forward and reverse primers across pool types.

Supplementary Tables

Supplementary Table 1. Patient demographics.

Demographic	Value
Patients (n)	30'782
Female patients (n)	14'613
Pediatric patients <18 years (n)	6'436
Minimal patient age (y)	1
Maximal patient age (y)	106
Median patient age (y)	62

Supplementary Table 2. Sample characteristics.

Sample type	Number (n)
Total samples	48'897
Nasopharyngeal swab	42'931
Bronchoalveolar lavage	3'961
Nasal swab	1'027
Throat swab	636
Tracheal/bronchial secretion	244
Sputum	98

Supplementary Table 3. RSV A subtype distribution per season.

Season	Age	A.D.1	A.D.1.5	A.D.1.6	A.D.1.7	A.D.2.1	A.D.3	A.D.3.1	A.D.5	A.D.5.1	A.D.5.2	A.D.5.3
2021–2022	≥18	0	0	0	0	0	1	0	2	0	0	0
2022–2023	≥18	1	0	0	0	0	0	0	0	0	0	0
2023–2024	≥18	9	0	2	0	1	7	2	2	7	2	1
2023–2024	<18	17	2	1	1	1	14	2	0	19	2	0

Supplementary Table 4. RSV B subtype distribution per season.

Season	Age	B.D.4.1.1	B.D.E.1
2019–2020	≥18	1	0
2021–2022	≥18	0	1
2022–2023	≥18	1	18
2023–2024	≥18	1	4
2023–2024	<18	0	3