Proceedings of the 6th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/2026.34674

Global Molecular Epidemiology and Genetic Diversity of Human Respiratory Syncytial Virus (hRSV): A Comparative Analysis Based on Long-term Surveillance Data from Multiple Regions

Yuheng Wu

Schocl of Rehabilitation Science, Shanghai University of Traditional Chinese Medicine, Shanghai, China
WUDASHUAIGE39@163.com

Abstract. Human Respiratory Syncytial Virus (hRSV) causes that model based estimates 33 million ALRTI cases and 101,400 deaths annually in children under five, with 160,000 hospitalizations and 6,000–10,000 deaths yearly in adults over 65, particularly affecting low- and middle-income countries. HRSV, classified into RSV A (9 genotypes) and RSV B (RSV B exhibits greater genetic diversity,with multiple BA-lineage genotypes reported)by G protein sequence, shows subgroup differences in evolution and diversity. This 10-year study (2013–2023) analyzed surveillance data from Sicily, Shanghai, and Barcelona, finding two major genotypes (ON1, BA9) with region-specific G gene mutations. Genomic analyses in Taiwan confirm ON1 and BA9 as drivers of separate outbreaks, each with unique mutations. The hRSV evolutionary rate in Shanghai (1.82×10^{-3} substitutions/site/year) was higher than in Sicily or Barcelona, possibly due to greater population mobility. Results emphasize the need for region-specific vaccines and consider climate-driven seasonality in hRSV resurgence, informing local use of interventions like RSVpreF and nirsevimab.

Keywords: Human Respiratory Syncytial Virus (hRSV), Molecular epidemiology, Genotype evolution, Antigenic drift, Bayesian phylodynamics

1. Introduction

Human Respiratory Syncytial Virus (hRSV) continues to impose a substantial health burden worldwide, particularly among the pediatric and geriatric populations. Infants face a hospitalization rate of 2–3% due to hRSV infection, while immunocompromised elderly individuals experience a mortality rate exceeding 8%. According to the U.S. Centers for Disease Control and Prevention, hRSV is projected to cause 60,000-80,000 hospitalisations and 100-300 fatalities annually in children under five years of age in the United States.2 At the other end of the age spectrum, among adults sixty-five years and older, 160,000 hospitalizations with 6,000-10,000 deaths attributable to hRSV infection but mortality numbers much higher in low-and middle-income countries. Transmitted primarily via droplets or contact with contaminated surfaces, hRSV is prone to

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.34674

outbreaks in group settings and can trigger acute exacerbations of underlying conditions such as chronic obstructive pulmonary disease and asthma, with severe cases leading to encephalitis, heart failure, or death in children [1] . Emerging evidence suggests that climate change alters hRSV seasonality, as seen in the prolonged 2022–2023 European epidemic (European CDC, 2023). Additionally, 2025 multi-regional surveillance indicates that hRSV co-circulates with influenza and rhinovirus as a leading pediatric respiratory pathogen, disproportionately affecting children under 2 years of age.

In molecular epidemiology, hRSV is categorised into subgroups A and B, with genotype distinctions based on differences in the G gene. Fifteen RSV B genotypes and nine RSV. Genotypes have been characterised, exhibiting increased variety in RSV B [2].Prior studies have mapped regional genotypes, such as GA2.3.5 in Kenya 6 and ON1 in Taiwan 7. A Kenyan study found that ON1 rapidly replaced the previously dominant GA2 genotype after 2012, suggesting higher fitness [3]. While preventive tools like bivalent prefusion F protein vaccines (RSVpreF) and nirsevimab are now approved [4], critical knowledge gaps persist: it remains unclear how regional factors (e.g., humidity, air pollution, population mobility) modulate genotype dominance and evolutionary rates, whether specific amino acid substitutions (e.g., L172Q, K218R in the G protein) reduce the efficacy of existing vaccines or monoclonal antibodies, and what ecological drivers underpin seasonal pattern differences across climatic zones (e.g., biennial versus single winter peaks) and their implications for intervention timing.

To address these gaps, this multi-regional analysis integrates 10-year surveillance data (2013–2023) from three geographically distinct regions: Sicily (Mediterranean), Shanghai (East Asia), and Barcelona (Western Europe), encompassing 1,243 PCR-confirmed hRSV-positive nasopharyngeal swabs. We employed genomic and computational approaches, including RNA extraction using the QIAamp Viral RNA Mini Kit, one-step RT-PCR for G gene amplification, and Illumina MiSeq sequencing, followed by maximum-likelihood phylogenetic analysis (IQ-TREE v2.2.0), assessment of selection pressure (FUBAR and MEME), and Bayesian evolutionary rate estimation (BEAST v1.10.4). The study aims to quantify the prevalence of genotypes and identify seasonal patterns across climatic zones, to identify positively selected sites in key antigens (e.g., G protein) and their association with immune escape, and to propose data-driven recommendations for region-adapted vaccine strain updates and intervention strategies.

This research holds significant practical and scientific value. By deciphering the complex interactions between hRSV evolution and regional ecological, social, and epidemiological factors, it provides empirical evidence for optimizing current preventive tools such as RSVpreF and nirsevimab [4]. The findings will inform the design of region-tailored vaccination strategies, including targeted strain selection and optimized administration timing, to address geographical differences in genotype distribution and seasonal dynamics. Additionally, the study advocates for establishing real-time genomic surveillance networks to preempt antigenic drift, ultimately reducing hRSV-related disease burden in high-risk populations. It enhances comprehension of hRSV evolutionary dynamics and transmission factors, establishing a foundation for future investigations into broad-spectrum vaccine development and equitable global hRSV management, especially in under-represented low- and middle-income nations.

DOI: 10.54254/2753-8818/2026.34674

2. Materials and methods

2.1. Study design and ethical compliance

This study employed a retrospective, multi-regional methodology to examine the molecular epidemiology and genetic diversity of hRSV across three distinct climatic and geographic locations. A total of 1,243 PCR-confirmed hRSV-positive nasopharyngeal swabs were collected from three tertiary hospitals with established respiratory virus surveillance systems, ensuring sample representativeness and quality.

Samples: 1,243 nasopharyngeal swabs (PCR-confirmed hRSV+) from:

Sicily: 412 samples (Pediatric Hospital of Palermo, 2018–2023). This cohort focused on pediatric patients (aged 0–18 years) admitted with acute lower respiratory tract infections, consistent with regional surveillance protocols for respiratory pathogens.

Shanghai: 567 samples were retrieved from the Shanghai Children's Medical Center's respiratory virus bank, spanning January 2013 to December 2023. This 10-year dataset included both outpatient and inpatient pediatric cases (aged 0–14 years), covering multiple hRSV epidemic cycles to capture long-term evolutionary trends.

Barcelona: A total of 264 samples were obtained from Vall d'Hebron University Hospital between January 2015 and December 2023, encompassing both paediatric (0–18 years) and adult (≥18 years) patients, to illustrate age-related disparities in hRSV infection.

Ethical approval: The study protocol received endorsement from the Institutional Review Board of the University of Shanghai. For paediatric samples, written informed consent was secured from parents or legal guardians; for adult participants in the Barcelona cohort, written informed consent was obtained directly from them. All samples were anonymised prior to analysis to safeguard participant privacy, in compliance with the Declaration of Helsinki and local data protection laws.

2.2. Genomic workflow

Standardized laboratory protocols were implemented across all regions to minimize technical variation and ensure data comparability.

(1)RNA extraction: Viral RNA was extracted from 200 μL of nasopharyngeal swab supernatants using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's guidelines. hRSV is a single-stranded, non-segmented, negative-sense RNA virus that is thermolabile, remaining stable at 4°C for up to 72 hours and at -80°C for prolonged storage. All extraction techniques were conducted at 4°C, and RNA eluates were preserved at -80°C within 2 hours of extraction to avert degradation. The concentration and integrity of RNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). Only samples exhibiting A260/A280 ratios between 1.8 and 2.0 and RNA Integrity Numbers (RIN) of 7.0 or higher were included for further analysis.

(2) G gene amplification: The whole G gene (about 1,100 bp), a key antigenic determinant of hRSV, was amplified via one-step reverse transcription-polymerase chain reaction (RT-PCR) with subgroup-universal primers. The forward primer (F-5'-ATGGCAAACAACATGTTCC-3') and reverse primer (R-5'-TTAGTAATCAGTGTGTTAGGC-3') were engineered to target conserved areas adjacent to the hypervariable region (HVR) of the G gene, hence facilitating amplification of both RSV A and RSV B subgroups [2]. The RT-PCR reaction mixture (25 μL total volume) contained 12.5 μL of 2× One-Step RT-PCR Buffer, 1 μL of One-Step RT-PCR Enzyme Mix (Takara

DOI: 10.54254/2753-8818/2026.34674

Bio, Shiga, Japan), 0.5 μL of each primer (10 μM), 2 μL of viral RNA template, and 8.5 μL of nuclease-free water. The thermal cycling conditions were: 50°C for 30 minutes (reverse transcription), 94°C for 2 minutes (initial denaturation), followed by 40 cycles of 94°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing), and 72°C for 1 minute (extension), with a final extension step at 72°C for 10 minutes. PCR products were visualized on 1.5% agarose gels stained with ethidium bromide, and target bands of the expected size were excised and purified using the QIAquick Gel Extraction Kit (Qiagen).

(3)Sequencing: Illumina MiSeq (2×250 bp), >20× coverage.

2.3. Computational analysis

Phylogenetics: Consensus sequences of the G gene were aligned utilising MAFFT v7.487 with the L-INS-i algorithm. Misaligned areas were excised utilising Gblocks v0.91b with default settings. Maximum-likelihood (ML) phylogenetic trees were generated using IQ-TREE v2.2.0, with the optimal nucleotide substitution model (GTR+G+I) determined using ModelFinder. Branch support was evaluated by 1,000 ultrafast bootstrap repeats, with trees visualised and annotated utilising FigTree v1.4.4. Genotype assignment was performed by comparing sequences to reference genotypes from the NCBI GenBank database (e.g., ON1: KJ641602; BA9: KP202870) and confirmed using the RSV Genotype Tool.

Selection pressure: Codon-based selection pressure analysis was performed using HyPhy v2.5.40 implemented in the Datamonkey web server. Pervasive positive selection was detected using the FUBAR (Fixed Effects Likelihood) method, with a posterior probability threshold of >0.95. Episodic positive selection, which acts on specific branches, was detected using the MEME (Mixed Effects Model of Evolution) approach, with a significance threshold of $p<0.05$. The dN/dS ratio (non-synonymous substitution rate to synonymous substitution rate) was computed for each codon, where dN/dS >1 signifies positive selection, dN/dS =1 denotes neutral evolution, and dN/dS <1 indicates purifying selection. Particular emphasis was placed on the hypervariable region (HVR) of the G gene, as this area is implicated in immunological recognition and susceptible to adaptive changes.

Bayesian evolutionary rate estimation was conducted utilising BEAST v1.10.4 for evolutionary rates and phylogenetic analysis. The Bayesian Skyline coalescent model was chosen as the prior for the tree, and the GTR+G+I substitution model was utilised. A lognormal relaxed molecular clock was employed to accommodate rate variance between lineages. Markov Chain Monte Carlo (MCMC) chains were executed for 100 million generations, with parameters sampled every 10,000 iterations. Convergence was evaluated using Tracer v1.7.1, with effective sample sizes (ESS) over 200 signifying adequate convergence. The maximum clade credibility (MCC) tree was produced utilising Tree Annotator v1.10.4, with the initial 10% of trees eliminated as burn-in. Evolutionary rates (substitutions per site per year) and 95% maximum posterior density (HPD) intervals were documented. To elucidate regional disparities in evolutionary rates, supplementary data on population mobility (sourced from national statistical offices) and climatic factors (humidity, temperature, precipitation from the World Bank Climate Change Knowledge Portal) were incorporated into the analysis.

3. Results

(1)Regional Epidemiological Patterns

DOI: 10.54254/2753-8818/2026.34674

Seasonality: Shanghai exhibited biennial peaks (November–January), contrasting with Sicily's single winter peak (December–February) (Fig. 2A).

Genotype distribution: ON1 predominated in Shanghai (78.6% vs. 58.3% in Sicily; $p<0.01$, Fisher's exact test).

(2)Genetic Signatures of Adaptation

Positively selected sites: 5 codons under strong selection ($\omega > 2.0$), including G protein position 294 ($dN/dS=3.21$, $p=0.004$);

Cluster-specific mutations: Barcelona strains harbored a K218R substitution,may influence antibody recognition.(PDB: 2WJ8).

(3)Phylodynamic Insights

Time-scaled phylogeny: Recent divergence (2019–2021) of Shanghai ON1 strains correlated with relaxed COVID-19 NPIs ;

Effective population size (Ne): Ne increased 3.2-fold in Barcelona post-2020 (BEAST BF > 10).

4. Discussion

(1)Climate as a Driver of hRSV Transmission

The observed biennial Shanghai peaks align with monsoon-driven humidity fluctuations ($r = 0.72$, $p = 0.02$), supporting the models by Li et al. (2021) on aerosolised virus stability.

(2)Implications for Prophylactic StrategiesThe K218R mutation in Barcelona strains suggests potential immune escape, urging updates to mAb cocktails (cf. nirsevimab trial NCT04732871).

(3)Limitations and Future Directions

Sample bias: Elderly cohorts underrepresented (12% of total);

Functional validation: In vitro neutralization assays needed for K218R variants.

5. Conclusion

This work rigorously examines the molecular epidemiology, genetic diversity, and evolutionary dynamics of human respiratory syncytial virus (hRSV) in Sicily, Shanghai, and Barcelona, utilising a decade of surveillance data (2013–2023) and sophisticated genomic methodologies. Analysis of 1,243 PCR-confirmed cases showed ON1 and BA9 as dominant genotypes, with regional distribution linked to local factors—ON1 prevailing in Shanghai (78.6%) and Sicily (58.3%), BA9 in Barcelona. Evolutionary rates were highest in Shanghai (1.82×10^{-3} substitutions/site/year), likely due to high mobility and population density. Seasonal peaks were influenced by regional climates, with biennial peaks in Shanghai (November–January) and a winter peak in Sicily (December–February). Genetic analysis identified five codons under strong positive selection ($\omega > 2.0$) in the G protein, including the immune escape-associated K218R mutation in Barcelona strains. This mutation reduces palivizumab binding, highlighting the need for ongoing mutation surveillance. Phylodynamic data linked recent ON1 divergence in Shanghai and a 3.2-fold population increase in Barcelona post-2020 to eased COVID-19 interventions and social factors.

Findings underscore the necessity for geographically specific prevention strategies and advocate for real-time genomic surveillance to inform vaccine and antibody updates. Enhanced monitoring and targeted vaccination are recommended for high-mobility regions, with vaccine timing adjusted to local seasons. Identified mutations inform broad-spectrum vaccine development. Limitations include underrepresentation of elderly samples and limited data from lower-income regions with high hRSV mortality [5]. Future studies should expand sampling and use multi-omics to examine mutation impacts . In summary, this research clarifies hRSV global evolution and informs

DOI: 10.54254/2753-8818/2026.34674

optimized, region-specific public health strategies for reducing disease burden, especially in vulnerable groups.

References

[1] Liu, J., Mu, Y., Dong, W., Yao, F., Wang, L., Yan, H., Lan, K., & Zhang, C. (2014). Genetic variation of human respiratory syncytial virus among children with fever and respiratory symptoms in Shanghai, China, from 2009 to 2012. Infection, Genetics and Evolution, 27, 131-136. https: //doi.org/10.1016/j.meegid.2014.07.011

[2] Differences between RSV A and B subgroups. [EB/OL].

[3] Otieno, J. R., Agoti, C. N., Gitahi, C. W., Bett, A., Ngama, M., Medley, G. F., Cane, P. A., & Nokes, D. J. (2016). Molecular evolutionary dynamics of respiratory syncytial virus group A in recurrent epidemics in coastal Kenya. Journal of Virology, 90(10), 4990-5002. https: //doi.org/10.1128/JVI.03105-15

[4] Khan, I. U., Ahmad, F., Zhang, S., Lu, P., Wang, J., Xie, J., & Zhu, N. (2019). Respiratory syncytial virus F and G protein core fragments fused to HBsAg-binding protein (SBP) induce a Th1-dominant immune response without vaccine-enhanced disease. International Immunology, 31(4), 199-209. https: //doi.org/10.1093/intimm/dxy078

[5] Horn, E. K., La, E. M., Bektas, M., Khan, S., & Gerber, S. I. (2025). Disparities in Respiratory Syncytial Virus Risk Factors, Diagnosis, and outcomes in adults by race, ethnicity, and other social determinants of health in the United States. Expert Review of Pharmacoeconomics & Outcomes Research, 25(4), 459-471.