Disease Burden and Subgroup Replacement of Respiratory Syncytial Virus (RSV) within the Respiratory Virome Landscape: A 24-Month Surveillance of Hospitalized Patients in Vietnam (2024–2025) Using Multiplex Real-time PCR Assay

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INTRODUCTION

Respiratory Syncytial Virus (RSV) is one of the primary causes of acute respiratory infections worldwide; yet its impact on the broader respiratory pathogens during subgroup replacement events is inadequately comprehended. A comprehensive assessment of this viral dominance is required in Vietnam due to seasonal surges and altering lineages. This study aims to quantify the ecological impacts of the transition of RSV subgroups in the respiratory system of hospitalized patients.

METHODOLOGY

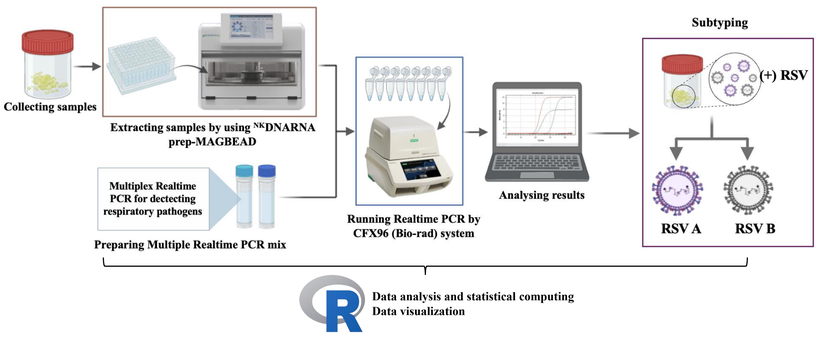

Figure 1. *Experimental workflow for respiratory pathogen surveillance. Schematic overview of the processing pipeline: Clinical sample collection is followed by automated nucleic acid extraction (NKDNARNA prep-MAGBEAD). High-throughput screening for respiratory pathogens is performed via multiplex real-time PCR (Bio-Rad CFX96 system), followed by confirmatory molecular subtyping into RSV-A and RSV-B. Statistical analysis and epidemiological visualizations are executed in R.*

RESULTS AND DISCUSSION

1. Temporal Dynamics and Significant Shifts in Respiratory Pathogen Distribution Over a 24-Month Surveillance Period

Table 1. Distribution of Respiratory Pathogens (2024-2025)

Comparative analysis of prevalence with statistical significance

Pathogen	2024 (N=2149) (n, %)	2025 (N=2862) (n, %)	Total (N=5011) (n, %)	p-value	Sig
Rhino	541 (25.2%)	735 (25.7%)	1276 (25.5%)	0.694	ns
RSV	373 (17.4%)	602 (21.0%)	975 (19.5%)	**0.001**	**
FluA	376 (17.5%)	556 (19.4%)	932 (18.6%)	0.085	ns
PIV-3	241 (11.2%)	390 (13.6%)	631 (12.6%)	**0.011**	*
AdV	194 (9.0%)	341 (11.9%)	535 (10.7%)	**0.001**	**
BoV	237 (11.0%)	293 (10.2%)	530 (10.6%)	0.378	ns
MeaV	277 (12.9%)	201 (7.0%)	478 (9.5%)	**< 0.001**	***
SARS-CoV-2	159 (7.4%)	174 (6.1%)	333 (6.6%)	0.067	ns
hMPV	96 (4.5%)	110 (3.8%)	206 (4.1%)	0.281	ns
FluB	55 (2.6%)	40 (1.4%)	95 (1.9%)	**0.003**	**
PIV-1	28 (1.3%)	44 (1.5%)	72 (1.4%)	0.549	ns
PIV-2	25 (1.2%)	30 (1.0%)	55 (1.1%)	0.784	ns
FluC	2 (0.1%)	49 (1.7%)	51 (1.0%)	**< 0.001**	***
HKU1	4 (0.2%)	16 (0.6%)	20 (0.4%)	**0.042**	*

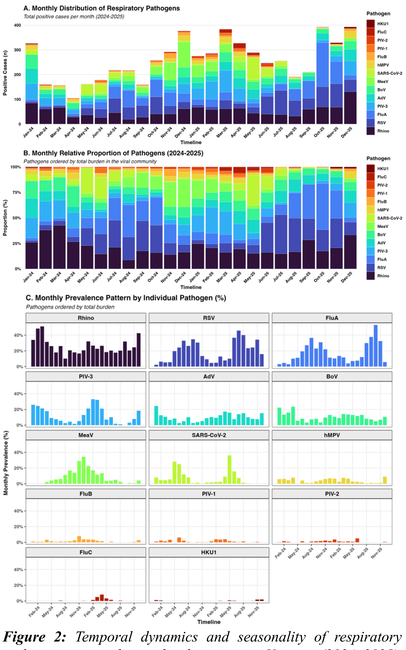

Figure 2: *Temporal dynamics and seasonality of respiratory pathogens among hospitalized patients in Vietnam (2024–2025). (A) Monthly Distribution of Positive Cases: Stacked bar chart representing the absolute number of positive cases (n) per month. Pathogens are color-coded and ordered by total prevalence, with dominant drivers such as Rhinovirus and RSV positioned at the base of the columns to facilitate trend tracking. (B) Relative Proportion of Pathogens (100% Stacked Bar): This panel illustrates the monthly changes in the viral community composition. The expansion of the RSV (dark blue) sector in late 2025 highlights its ecological dominance and potential interference with other circulating viruses during peak seasonality. (C) Monthly Prevalence Pattern by Individual Pathogen: Faceted bar charts displaying the monthly prevalence rate (%) for each of the 14 monitored viruses. All sub-panels utilize a fixed Y-axis to allow for a direct comparison of the clinical burden between major pathogens (e.g., RSV and Rhinovirus) and lower-prevalence agents (e.g., HKU1 or SARS-CoV-2).*

2. The Transition from RSV-A to RSV-B Dominance Correlates with Fluctuations in the Shannon Diversity Index

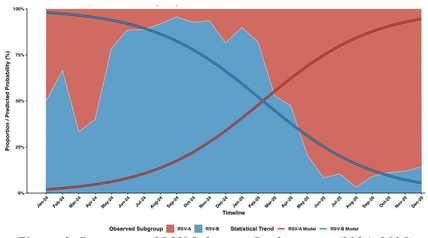

Figure 3*. Dynamics of RSV Subgroup Replacement (2024–2025). Integration of longitudinal surveillance data and statistical modeling. Shaded areas represent observed monthly proportions of RSV-A (red) and RSV-B (blue) in clinical samples; solid lines depict the logistic regression model (S-curves) confirming a statistically significant subgroup replacement over time.*

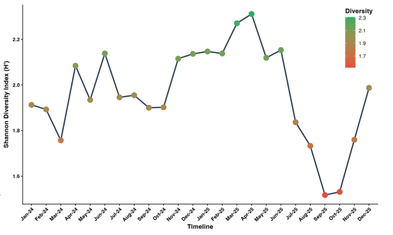

Figure 4: *Respiratory Virome Diversity Dynamics (2024–2025). Temporal tracking of community complexity via Shannon Diversity Index (H'). A local minimum in Sep–Oct 2025 directly synchronizes with the RSV-A prevalence peak, showing a moderate inverse correlation (rho = -0.38, p = 0.066). High-baseline pathogens (e.g., Rhinovirus) maintained ecosystem diversity above 1.5. Color gradient represents the transition from low-diversity dominance (red) to high-diversity evenness (green).*

3. Age-Specific Vulnerability and Distinct Bacterial-Viral Co-infection Profiles Across Surveillance Years

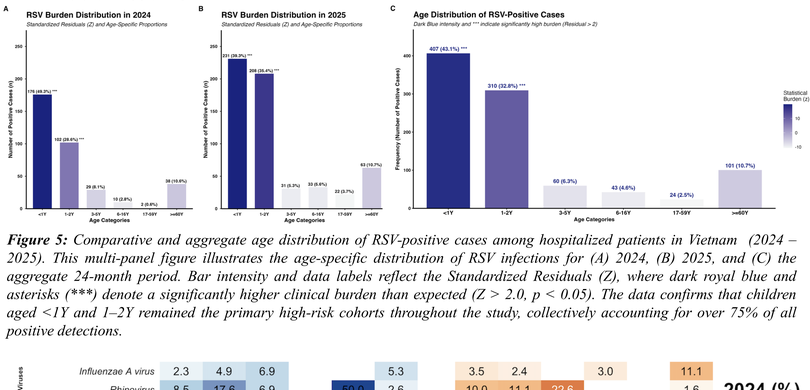

Figure 5: *Comparative and aggregate age distribution of RSV-positive cases among hospitalized patients in Vietnam (2024 – 2025). This multi-panel figure illustrates the age-specific distribution of RSV infections for (A) 2024, (B) 2025, and (C) the aggregate 24-month period. Bar intensity and data labels reflect the Standardized Residuals (Z), where dark royal blue and asterisks (***) denote a significantly higher clinical burden than expected (Z > 2.0, p < 0.05). The data confirms that children aged <1Y and 1–2Y remained the primary high-risk cohorts throughout the study, collectively accounting for over 75% of all positive detections.*

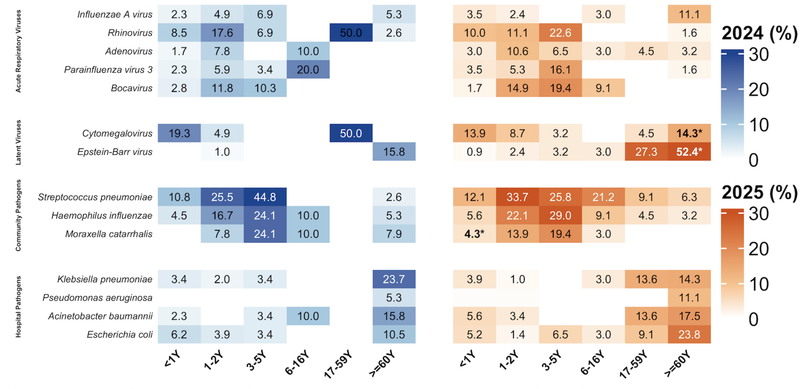

Figure 6: *Age-stratified pathogen prevalence comparison in RSV-positive cases among hospitalization patients (2024 - 2025). This dual-panel heatmap illustrates the prevalence (%) of 14 secondary pathogens among RSV-positive patients, segmented into (left) 2024 and (right) 2025 cohorts. Pathogens are organized into four functional clusters: Acute Respiratory Viruses, Latent Viruses, Community Pathogens, and Hospital Pathogens; separated by distinct horizontal gaps to enhance ecological differentiation. Cell values indicate the mean prevalence, with asterisks (*) in the 2025 panel denoting statistically significant shifts from the previous year based on Fisher's Exact Test (p < 0.05).*

CONCLUSION

The shift to RSV-A predominance in Vietnam is marked by an increased significant effect on the respiratory ecology. While infants represent the most vulnerable demographic in terms of quantity, the elderly face an extremely complicated microbial environment during peak seasons. These findings highlight the necessity for genotype-specific surveillance and age-stratified management methods in hospital environments to minimize the multifaceted burden of RSV.

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