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RESEARCH ARTICLE

Epidemic Outbreak of Respiratory Syncytial Virus Infection After the end of the Zero-COVID-19 Policy in China: Molecular Characterization and Disease Severity Associated With a Novel RSV-B Clade

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Keywords: disease severity | genetic diversity | outbreak | out-of-season epidemic | respiratory syncytial virus

ABSTRACT

The resurgence of respiratory syncytial virus (RSV) has become a major concern recently. This study aimed to describe the temporal dynamics, genotype variability and disease severity of RSV infection after the end of the Zero-COVID-19 policy in Beijing, China. A total of 905 patients were positive for RSV at National Center for Respiratory Medicine in Beijing from November 2019 to April 2024. Of these, 238 positive samples from different patients were successfully sequenced, 96 of which were identified as the RSV-A and 142 as the RSV-B. Phylogenetic analyses were performed to investigate genetic diversity. The first surge of RSV infection after the end of the Zero-COVID-19 policy was quite intense and occurred out of season, mainly affecting children. The subsequent RSV outbreak had a significant impact among adults. RSV cases were caused by various clades and a new clade B.D.E.1 was identified as the main cause of the epidemic outbreak in adults. Pneumonia in immunocompromised hosts caused by clade B.D.E.1 was more common compared to other clades. Accumulation of substitutions in clade B.D.E.1 could confer a fitness advantage in vivo. However, there was no statistical difference in clinical outcomes between patients infected by clade B.D.E.1 and those infected by other RSV clades. This study addressed the timing and trends of the RSV infections, focusing on epidemic outbreaks, molecular characterization, and disease severity associated with a novel clade B.D.E.1. A more effective prevention strategy for RSV infections in childhood, immunosuppressed adults and elderly might be warranted.

Abbreviations: CCD, central conserved domain.; COVID-19, corona virus disease 2019; HVR, highly variable region; ICU, intensive care unit; PCR, polymerase chain reaction; RSV, respiratory syncytial virus;.

Yulin Zhang and Dongya Pu contributed equally to this study.

1 | Introduction

Respiratory syncytial virus (RSV) is a major pathogen responsible for causing acute lower respiratory tract infections in young children worldwide [1]. It is estimated that the global incidence of RSV-associated lower respiratory tract infection exceeds 30 million cases in children under the age of five, leading to approximately 3.2 million hospitalizations [1]. However, RSV infection also represents a significant disease burden and could cause acute respiratory illness and trigger exacerbations of cardiopulmonary diseases in elderly individuals, the outcomes of which are significantly worse compared to infants [2–4].

RSV can be categorized into two groups, A (RSV-A) and B (RSV-B), based on their reactivity to monoclonal antibodies. Differences in virulence among various RSV genotypes have been reported, although these findings remain contentious [5–7]. As of now, multiple genotypes of respiratory syncytial virus (RSV) can be further categorized, including at least 15 RSV-A and 30 RSV-B subtypes, based on the variability of the highly variable region (HVR) sequences. These HVR sequences are characterized as highly glycosylated 'mucin-like' domains that are rich in proline, serine, and threonine [8, 9]. Among these, ON1 and BA9, characterized by a duplication in the HVR of the G gene, have demonstrated a selective advantage and circulated globally in recent years [10]. Recently, a comprehensive naming system for RSV has been suggested, which could improve the understanding of its molecular epidemiology and evolution worldwide [11, 12]. During the pre-pandemic period, the seasonal epidemiology of RSV was characterized by an unclear alternation between RSV-A and RSV-B [5]. However, the genetic evolution of RSV may have been affected by the COVID-19 pandemic. The decline in infections could have resulted in the disappearance of lineages that existed before the pandemic and the appearance of novel RSV clades, similar to trends observed in other countries [13–15].

The incidence of acute respiratory tract infections in our country has significantly increased following the end of Zero-COVID-19 policy in Jan 8, 2023, with multiple RSV lineages circulating after the pandemic [14]. Notably, two novel imported clades, A.D.5.2 and B.D.E.1, were detected for the first time in China during the COVID-19 pandemic [16]. It remains unclear whether the newly identified RSV clades are associated with the epidemic patterns and disease severity. This study aimed to describe the circulation patterns and genetic diversity of RSV, as well as to compare the clinical characteristics of RSV infections among the different clades.

2 | Materials and Methods

2.1 | Study Population and Clinical Data Collection

We carried out a retrospective study encompassing all respiratory specimens (nasopharyngeal swabs, bronchoalveolar lavage fluid, and sputum specimens) that tested positive for RSV in patients of all ages from November 2019 to April 2024 at National Respiratory Medical Center in Beijing. All electronic medical records related to the study were collected from all participants. The data included demographic details, comorbid illnesses, pneumonia, coinfections, intensive care unit (ICU) admission, and clinical outcomes. This study was approved by the China-Japan Friendship Hospital Medical Ethical Committee (2024-KY-073).

2.2 | RSV Detection and Sequence Analysis

RSV infection was confirmed by analysis of nasopharyngeal, BALF, or sputum specimens using the SureX 13 Respiratory Pathogen Multiplex Detection Kit (Cat. No. 1060099, Ningbo Health Gene Technology), the Xpert® Xpress Flu/RSV tests (Cepheid) and rapid antigen tests for RSV (Hangzhou Genesis Biodetection & Biocontrol Co. Ltd). Realtime PCR methods from Liferiver (Shanghai, China) were utilized for the subgrouping of RSV. In addition, the G genes of the RSV-A and RSV-B were amplified by nested PCR and PCR products were then sequenced using Sanger sequencing. The primers used in this study are listed in Table S1.

2.3 | Phylogenetic Analysis and Amino Acid Analysis

A total of 96 RSV-A G genes and 142 RSV-B G genes were obtained and used for further analysis. The phylogenetic trees of RSV-A and RSV-B were constructed with IQ-TREE using maximum likelihood, and the bootstrap values were 1000. All phylogenetic trees were visualized by the Interactive Tree Of Life (https://itol.embl.de). GenBank accession numbers of RSV-A and RSV-B reference sequences were KC297374.1 and MF445739.1. We used Nextclade v3.10.0 (https://clades.nextstrain.org/) to perform the genomic alignment, clade assignment, and genetic variation annotations of G genes. Both of the translated sequences of clades A.D.5.2 and A.D.3 were aligned to the sequence of clade A.D.1 (PQ822247 in this study), respectively. The translated sequences of clades B.D.E.1 and B.D.E.2 were aligned to the sequence of clade B.4.1.1 (PQ822338 in this study). In addition, N- and O-linked glycosylation sites were predicted using the NetNGlyc and NetOGlyc service available at https://services.healthtech.dtu.dk.

2.4 | Statistical Analysis

In conducting the statistical analysis for the tables, we selected appropriate statistical methods based on the type of variables and the research objectives. Chi-squared was used to test the statistical difference for categorical variables. We calculated the actual frequencies and expected frequencies for each categorical variable, then used the chi-squared formula to compute the chi-squared statistic and obtained the corresponding p-value from tables or statistical software. The student t-test was used to compare quantitative variables. We calculated the means, standard deviations, and sample sizes for the two groups, then used the t-test formula to compute the t-statistic and obtained the corresponding p-value from tables or statistical software. If the p-value is less than or equal to 0.05, we conclude that there is a statistically significant difference. All probabilities were

2-tailed, with statistical significance defined as $p \leq 0.05$. All analyses were performed using PASW Statistics software, version 25.0.

3 | Results

3.1 | RSV Monthly Distribution

From November 2019 to April 2024, 905 patients were positive for RSV in our center. Of these, 472 samples from different RSV-infected patients, including 162 RSV-A and 310 RSV-B samples, were collected and analyzed. As shown in Figure 1A and Figure 1B, the prevalence of RSV began in November 2019, and since the outbreak of COVID-19 in December 2019, the positive rate of RSV has significantly decreased from December 2019 to March 2020 (the RSV epidemic season). From November 2020 to March 2021, only sporadic cases of RSV were reported. From November 2021 to March 2022, RSV epidemic patterns were higher than those in previous years (Figure 1A).

From November 2022 to March 2023, the end of the Zero-COVID-19 policy in China was implemented, and during this period, an outbreak of COVID-19 occurred and no RSV-positive samples were detected. It is worth noting that between April 2023 and June 2023, RSV showed a small outbreak during the non-epidemic season, primarily in children. Moreover, RSV has been widespread among adults since November 2023 (Figure 1A and Figure 2A).

Out of 905 patients, the genotypes of 433 patients were unknown or undetected, representing 47.8%. RSV-A accounted for 162 out of 905 (17.9%), while RSV-B accounted for 310 out of 905 (34.3%) (Figure 1B). From November 2019 to December 2022 (P1), RSV-A and RSV-B were co-prevalent, with the positivity rate of RSV-A being higher than that of RSV-B (26.8% vs 22.0%, $p > 0.05$). Whereas, from January 2023 to April 2024 (P2), RSV-B prevailed in contrast to RSV-A (37.9% vs 15.3%, $p < 0.05$) (Figure 1B and Table 1).

3.2 | Demographic Characteristics of RSV-Positive Cases Identified Before and After the End of the Zero-COVID-19 Policy

Of 17568 respiratory specimens tested from P1 (before the end of the Zero-COVID-19 policy), 249 (1.4%) samples were RSV positive. The absolute percent of samples testing positive for RSV increased to 4.1% (945/23258) from P2 (after the end of the Zero-COVID-19 policy). The coinfection rate from P2 was approximately four times that from P1 (12.9% vs 4.4%, $p < 0.05$). The most common coinfecting virus was rhinovirus (3/9, 33.3%) from P1 and influenza A virus (28/90, 31.1%) from P2, respectively. From P1, a total of 205 patients were positive for RSV, with 104 males accounting for 50.7% (104/205) of cases. The average percentage of males from P2 (60.9%, 426/700) was slightly greater than that from P1, and the difference was significant ($p < 0.05$). The mean age of RSV-positive subjects from P2 was higher (48.1 years vs 39.8 years, $p < 0.05$) compared to that from P1. Overall, the rate of RSV positivity in children from P1 was higher than that from P2 (41.0% vs 26.1%, $p < 0.05$), whereas, for adults, it was the opposite (59.0% vs 73.9%, $p < 0.05$) (Table 1).

For children, the rates of RSV positivity in children aged 1–5 years from P1 were higher than that from P2 (75.0% vs 57.4%, $p < 0.05$), while the rates of RSV positivity in children aged 6–17 years from P1 were lower than that from P2 (0.0% vs 15.3%, $p < 0.05$). The rate of RSV positivity in children aged less than 1 years showed no difference between P1 and P2 ($p > 0.05$). Whereas for adults, patients aged over 60 years were the main population infected by RSV both from P1 (80/121, 66.1%) and again from P2 (313/517, 60.5%). The rate of RSV positivity remained similar among adults aged 18–60 years and those over 60 years old from P1 and P2 ($p > 0.05$) (Table 1).

3.3 | Genotyping and Phylogenetic Analysis of RSV

A total of 238 positive samples from different patients were successfully sequenced, 96 of which were identified as the RSV-A and 142 as the RSV-B. For phylogenetic analyses, the RSV-A data set contained 60 sequences from P2 and 36 sequences from P1; the RSV-B data set contained 105 sequences from P2 and 37 sequences from P1. The results indicated that all sequenced samples classified as RSV-A belong to the ON1 genotype. All RSV-A sequences carried the 72-nucleotide insertion in the HVR of the G gene; however, they were evolutionarily distant from the prototype ON1 strain. All sequences clustered with clades A.D.1, A.D.2.2.1, A.D.3, A.D.5.2, interestingly, 15 sequences from P2 grouped into a new clade A.D.5.2 (Figure 2B). Furthermore, both clade A.D.3 and A.D.5.2 could have evolved from clade A.D.1. Notably, the new clade A.D.5.2 was the closest to clade A.D.1, while clade A.D.3 spread more widely in our research (Figures 2B,C,D and 3A).

All RSV-B sequences carried a 60-nucleotide insertion in the HVR of the G gene and clustered with clades B.D.4.1.1, B.D.E.1, B.D.E.2 (Figure 3B). Both 22 sequences from P1 and the 18 sequences from P2 obtained in this study clustered with clade B.D.4.1.1, while all 13 sequences identified from P1 clustered with clade B.D.E.2. Two sequences from P1 and 87 sequences from P2 clustered with clade B.D.E.1, which was recognized as a newly imported lineage of RSV-B strains (Figures 2B and 3B). Additional analysis of RSV-B sequences suggested that clade B.D.E.1 could have evolved from clade B.D.4.1.1 (Figure 3B).

3.4 | Amino Acid Analysis

To better explore amino acid substitutions, we compared the variations in sequences of clades A.D.3 and A.D.5.2 with those of clade A.D.1 in our study. As shown in Figures 4A and 4B, compared to the central conserved domain (CCD) of clade A.D.1, the N178G substitution occurred in A.D.3 rather than A.D.5.2, with a frequency of 77/77 (100.0%). In the mucin-like domain I, clade A.D.3, rather than A.D.5.2, had the P95S, T113I, A116D, V131D and R151H substitutions relative to A.D.1, with a frequency of more than 10.0%. In the mucin-like domain II, the P230T, T245A, H258Q, H266L, Y273H, P274L, G284S,

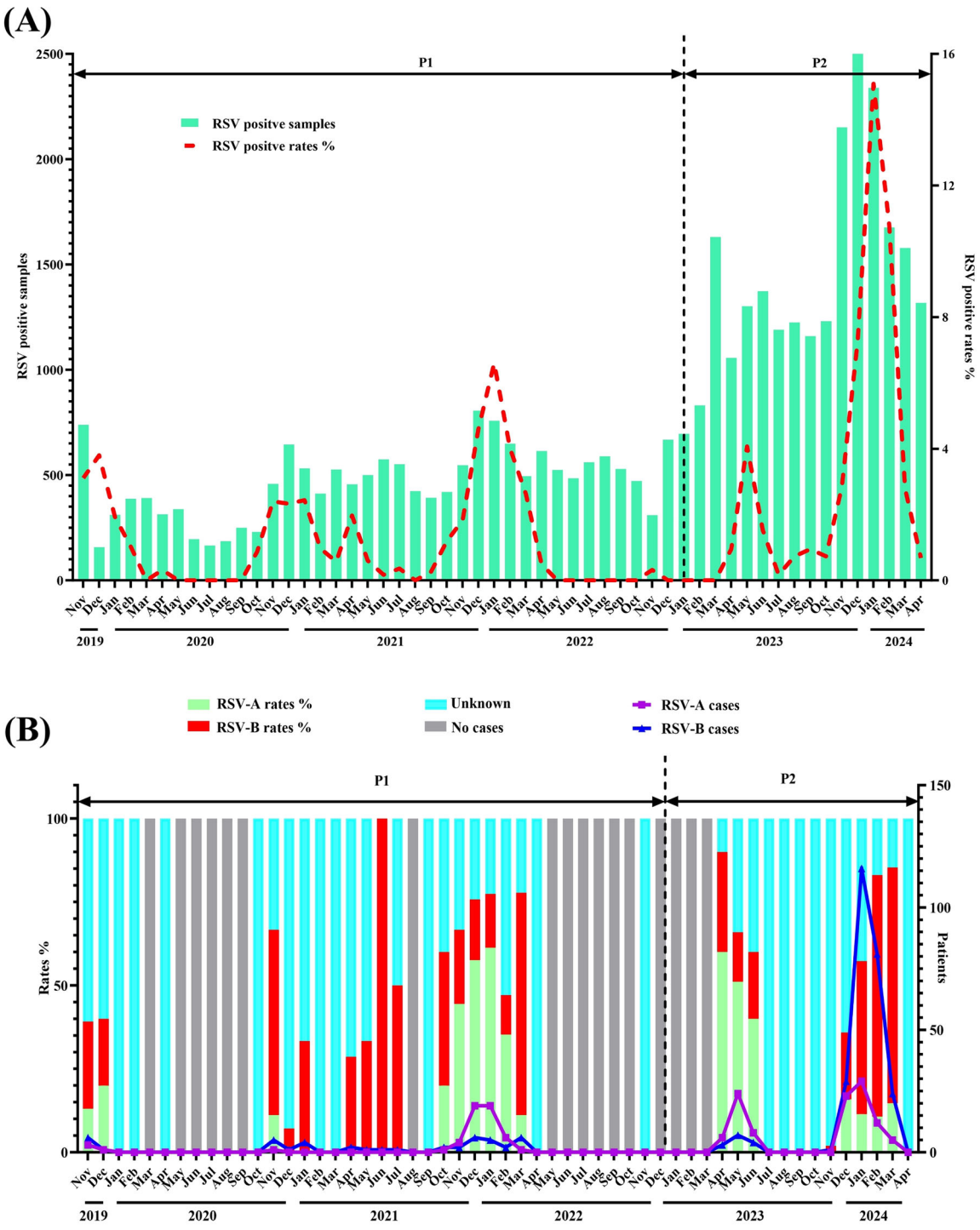

FIGURE 1 | Monthly distribution of RSV-positive specimens in patients with respiratory tract infection in Beijing from November 2019 to April 2024. P1 represents November 2019 to December 2022; P2 represents January 2023 to April 2024. (A) Monthly distribution of RSV-positive specimens. The numbers of RSV-positive specimens are shown in the green column diagram, and the positive rates of RSV are shown in the red broken line graph. (B) Monthly distribution of RSV-A and RSV-B positive patients. The numbers of RSV-A and RSV-B positive patients are shown in the line graph with purple for RSV-A and blue for RSV-B, respectively, and the proportions of RSV-A and B are shown in the column diagram with green for RSV-A and red for RSV-B, respectively. RSV, respiratory syncytial virus.

L289I, P298L, S299N, H304Y, P310L and A320T substitutions occurred in clade A.D.3, but not in clade A.D.5.2, compared to clade A.D.1, with an occurrence ratio of more than 10.0%.

During the evolution of RSV-B, the amino acid substitutions of G proteins in clade B.D.E.1 occurred in the mucin-like domain I and II compared with clade B.4.1.1. The heparin binding domain, responsible for binding to glycosaminoglycans on the cell surface and mediating infection, was located between amino acids 181 and 195 in the G protein of RSV strains isolated in this study, and it had no mutations. As described in Figures 4C and 4D, the substitutions in

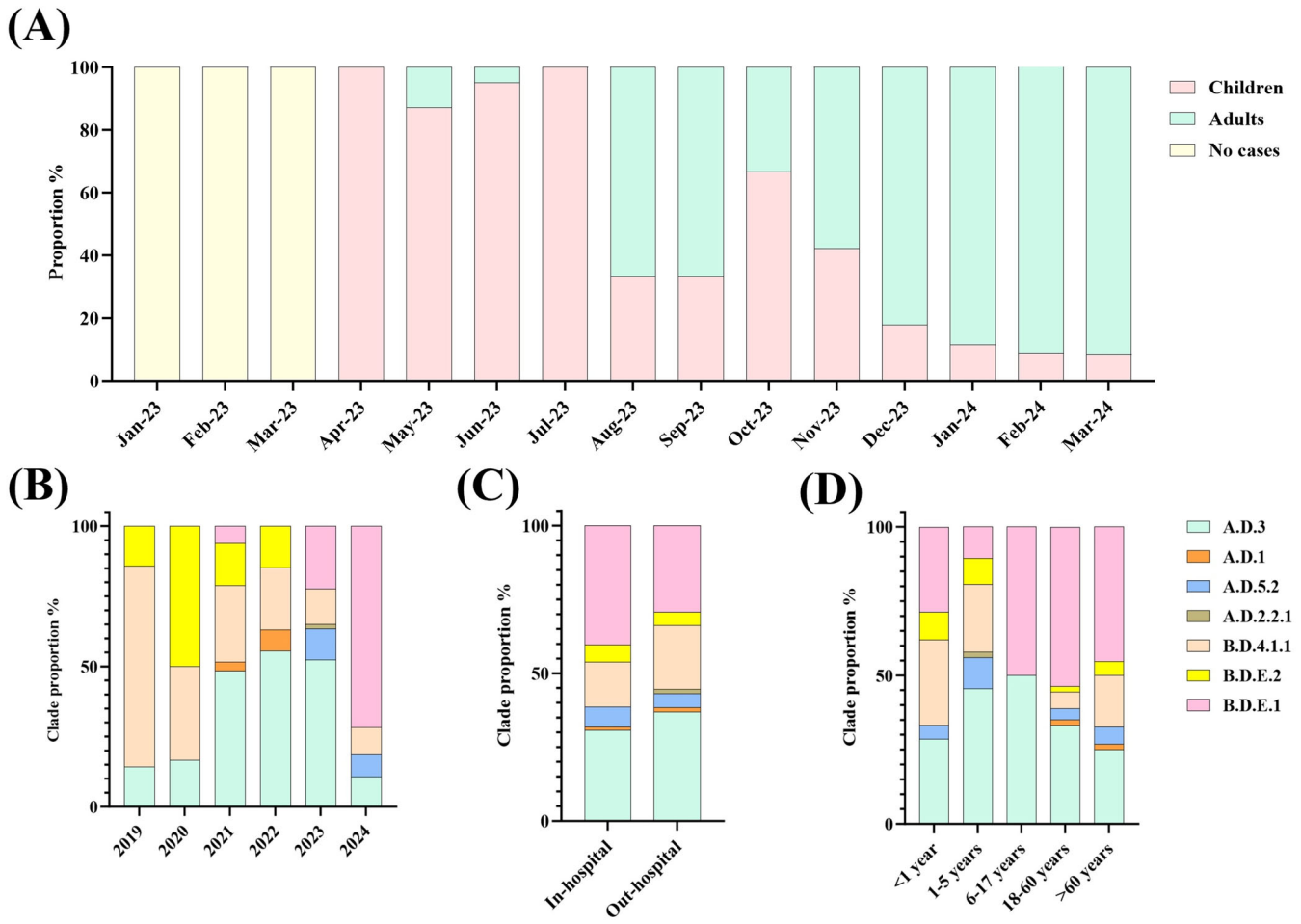

FIGURE 2 | Time, hospitalization and age distribution of RSV infection. (A) Monthly distribution of RSV infection in children and adults. (B) Year distribution of different RSV clades from November 2019 to April 2024. (C) Hospitalization distribution of different RSV clades from November 2019 to April 2024. (D) Age distribution of different RSV clades from November 2019 to April 2024.

mucin-like domain II in clade B.D.E.1 but not in clade B.D.E.2 included P214S, P221L, K256N, S265P, I268T, S275P and H277R, with a proportion of more than 10.0%. Additionally, in the mucin-like domain I, the S100G, T131A and I137T occurred only in clade B.D.E.1, with substitution ratios ranging up to 100% and the T117A occurred with a substitution ratio of 12/89 (13.5%). In addition, a specific mutation (N176S) in the CCD gene was observed in all 13 strains belonging to clade B.D.E.2, which were isolated exclusively before 2023 and, to our knowledge, no new strains with this mutation have been isolated since 2023 (Figure 4D).

Compared with clades A.D.1 and A.D.3, there were no considerable differences in the predicted O-glycosylationsites and NO-glycosylation sites in clade A.D.5.2. Compared with clade B.D.4.1.1 and B.D.E.2, the S100G, T131A and S275P substitutions in sequences of clade B.D.E.1 resulted in the loss of three O-glycosylation sites. Furthermore, the T117A/I substitution in the sequences of 13 patients each resulted in the loss of one O-glycosylation site. In addition, all sequences carrying the K256N substitution in clade B.D.E.1 acquired a fourth predicted N-glycosylation site, whereas three additional N-glycosylation sites were conserved in both clades B.D.4.1.1 and B.D.E.2 (data not shown).

3.5 | Comparison of Clinical Characteristics Between Adults Infected by RSV Clade B.D.E.1 and Other Clades

The clinical data of the patients infected by the novel clades (A.D.5.2 and B.D.E.1) are summarized. Very few people are infected with clade A.D.5.2, thus clinical characteristics have not been analyzed in this study. The proportions of hospitalized patients and women infected with clade B.D.E.1 are similar to that of those without clade B.D.E.1 ($p > 0.05$). The mean age of RSV-positive subjects infected by clade B.D.E.1 was higher (55.1 years vs 37.5 years, $p < 0.05$). Eighty-five percent of patients infected with clade B.D.E.1 were adults, children accounted for only 14.6%. While children and adults comprised nearly 50% of the patients without clade B.D.E.1 (Table 2 and Figure 2D).

A total of 62 and 59 adult inpatients, infected with clade B.D.E.1 and without clade B.D.E.1 respectively, were selected for analysis of clinical characteristics and outcomes. There was no difference in underlying diseases, bacteria/fungus/viral confections, ICU admission and clinical outcomes between two cohorts ($p > 0.05$). As for the organ transplant recipients, patients with clade B.D.E.1 infection were higher than those

TABLE 1 | Demographic characteristics and subgroup distribution of RSV-positive cases identified from Nov 2019 to Dec 2022 and from Jan 2023 to Apr 2024.

RSV-positive samples	P1[a]	P2[b]	*p* value
RSV-positive samples	249/17568 (1.4%)	945/23258 (4.1%)	**0.000**
RSV-positive patients	205/905 (22.7%)	700/905 (77.3%)	**0.000**
RSV in coinfection	9/205 (4.4%)	90/700 (12.9%)	**0.001**
RSV/Rhinovirus	3/9 (33.3%)	11/90 (12.2%)	0.218
RSV/Influenza A virus	0/9 (0.0%)	28/90 (31.1%)	0.057
RSV/influenza B virus	1/9 (11.1%)	23/90 (25.6%)	0.578
RSV/Parainfluenza virus	0/9 (0.0%)	8/90 (8.9%)	1.000
RSV/Adenovirus	1/9 (11.1%)	7/90 (7.8%)	1.000
RSV/Mycoplasma pneumoniae	1/9 (11.1%)	3/90 (3.3%)	0.809
RSV/Chlamydia pneumoniae	0/9 (0.0%)	0/90 (0.0%)	—
RSV/Metapneumovirus	0/9 (0.0%)	7/90 (7.8%)	1.000
RSV/Coronavirus	2/9 (22.2%)	10/90 (11.1%)	0.661
RSV/Boca virus	1/9 (11.1%)	0/90 (0.0%)	0.091
Male	104/205 (50.7%)	426/700 (60.9%)	**0.010**
Mean age in years (SD)	39.8 (33.7)	48.1 (30.2)	**0.002**
Children	84/205 (41.0%)	183/700 (26.1%)	**0.000**
< 1 year	21/84 (25.0%)	50/183 (27.3%)	0.690
1–5 years	63/84 (75.0%)	105/183 (57.4%)	**0.006**
6–17 years	0/84 (0.0%)	28/183 (15.3%)	**0.000**
Adults	121/205 (59.0%)	517/700 (73.9%)	**0.000**
18–60 years	41/121 (33.9%)	204/517 (39.5%)	0.256
> 60 years	80/121 (66.1%)	313/517 (60.5%)	0.256
RSV-A-positive patients (%)	55/205 (26.8%)	107/700 (15.3%)	**0.000**
RSV-B-positive patients (%)	45/205 (22.0%)	265/700 (37.9%)	**0.000**

[a]P1 represents Nov 2019 to Dec 2022.
[b]P2 represents Jan 2023 to Apr 2024.

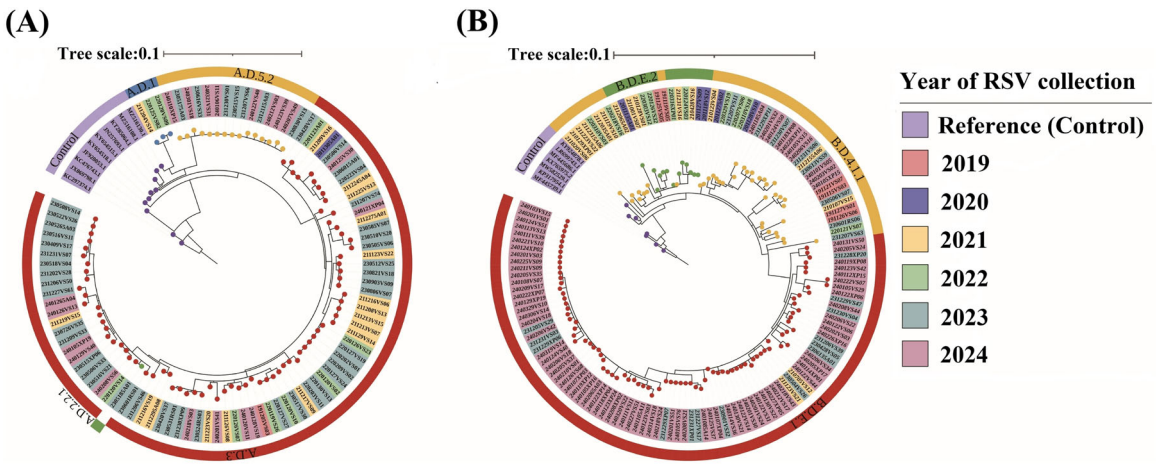

FIGURE 3 | Phylogenetic analysis of the G gene of RSV-A (A) and RSV-B (B) strains. The outer ring: clades of the G protein in RSV-A (A) and clades of the G protein in RSV-B (B); A: A.D.1, A.D.5.2, A.D.3, A.D.2.2.1; B: B.D.E.2, B.D.4.1.1, B.D.E.1. The inner ring: the names of clinical RSV strains from different patients collected in our laboratory at different time periods (e.g., "240105VS76 and 240105XP15", 240105 indicates that it was collected on January 5th, 2024; VS76 or XP15, which represents the laboratory test code). The genes and predicted protein sequences of each strain have been uploaded to NCBI. GenBank accession numbers of RSV-A and RSV-B reference sequences were KC297374.1 and MF445739.1. The phylogenetic trees of RSV-A and RSV-B were constructed with IQ-TREE using maximum likelihood, and the bootstrap values were 1000.

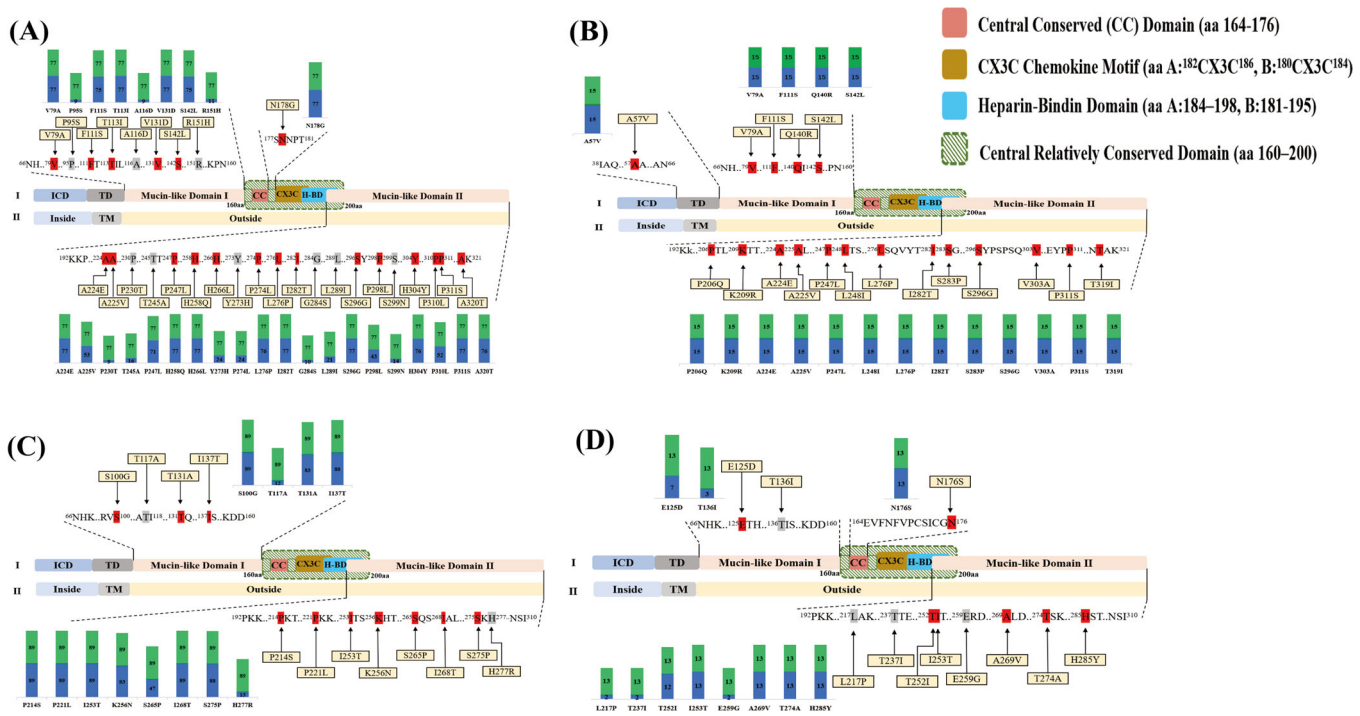

FIGURE 4 | RSV genome denoting nonsynonymous sequence polymorphisms. The frequency of these point mutations among viruses collected in China were present. I: Coding regions of RSV G protein reported in the literature; ICD: Intracellular Cytoplasmic Domain, TD: Transmembrane Domains. II: RSV G protein transmembrane domains predicted by TMHMM2.0; TM: Transmembrane. The amino acid sequences highlighted in red and gray indicate mutation sites. Specifically, the red-highlighted sequences have a mutation proportion exceeding 50%, while the gray-highlighted sequences have a mutation proportion ranging from 11.7% to 31.2%. The bar chart shows the number of patients at each mutation site and the total number of patients with RSV G protein detected. (A) A.D.3 vs A.D.1. (B) A.D.5.2 vs A.D.1. (C) B.D.E.1 vs B.D.4.1.1. (D) B.D.E.2 vs B.D.4.1.1.

without clade B.D.E.1 infection ($p < 0.05$). Furthermore, pneumonia in immunocompromised hosts caused by clade B.D.E.1 infection was more common compared to other RSV clades ($p = 0.05$) (Table 2). In addition, the loss of an N-glycosylation site resulting from T117A/I substitutions was more common in immunocompromised patients with pneumonia than those without it in this study (50.0% vs 10.9%, $p < 0.05$).

4 | Discussion

This study mainly described the RSV epidemics before and after the end of the Zero-COVID-19 policy in China. From P1 (before the end of the Zero-COVID-19 policy), standardized procedures for epidemic prevention and control in hospitals and health institutions could greatly lower the rate of nosocomial infections caused by RSV [17, 18]. The end of the Zero-COVID-19 policy took place in January 2023, during which a COVID-19 outbreak occurred in China. From P2 (after the end of the Zero-COVID-19 policy), the RSV epidemic emerged quite intensely, with an out-of-season outbreak in children and an epidemic outbreak in adults.

As indicated by previous studies, RSV infections were observed more frequently in adults after the end of the Zero-COVID-19 policy compared to those observed before, which warrants greater attention [19]. Moreover, clade B.D.E.1 predominated during the epidemic outbreak among adults in P2 (Figure 2D). Notably, the percentage of organ transplant recipients infected by clade B.D.E.1 was much higher than that of those infected by strains without clade B.D.E.1 (Table 2). Further analysis indicated that clade B.D.E.1 might have evolved from clade B.D.4.1.1, and it exhibited certain amino acid substitutions in the mucin-like domains I and II when compared to clade B.D.4.1.1. A previous study showed that a new RSV lineage characterized by an N64D substitution in the SH protein was associated with an outbreak of severe RSV-B infection in children [20]. Another research revealed that long shedding periods and the absence of immune selective pressure in immunocompromised hosts could allow the persistence of viruses that lack a part of the C-terminus of the G glycoprotein [21]. Whether the new substitutions carried by clade B.D.E.1 are linked to the outbreak of RSV infection among adults and are more prevalent in immunocompromised hosts remains to be further investigated.

It was found here that RSV-A prevailed over RSV-B from P1, whereas RSV-B occurred more frequently from P2 (Table 1). RSV-A sequences isolated from this study clustered with clade A.D.1, A.D.2.2.1, A.D.3, and A.D.5.2. Interestingly, a novel imported clade A.D.5.2 was identified in China after the COVID-19 pandemic, but it was only sporadic in a few patients. While clade A.D.3, characterized by the N178G substitution in the CCD, was the most prevalent in our center, a previous study suggested that the two conserved asparagines, N178 and N179, serve as epitopes for broadly neutralizing monoclonal antibodies [22, 23]. Therefore, the N178G mutation found in clade A.D.3 may weaken its binding ability to the corresponding antibodies, thereby enabling immune escape. Furthermore,

TABLE 2 | Demographic and clinical characteristics of RSV-positive cases infected with clade B.D.E.1 and none clade B.D.E.1.

Characteristics	Group		*p* value
	Clade B.D.E.1	Without clade B.D.E.1	
Study population	89	149	
Inpatients	70/89 (78.7%)	103/149 (69.1%)	0.111
Outpatients	19/89 (21.3%)	46/149 (30.9%)	0.111
Female	38/89 (42.7%)	71/149 (47.7%)	0.458
Male	51/89 (57.3%)	78/149 (52.3%)	0.458
Age/years	55.1 (26.8)	37.5 (33.7)	**0.000**
Children	13/89 (14.6%)	67/149 (45.0%)	**0.000**
< 1 year	6/13 (46.2%)	15/67 (22.4%)	0.075
1–5 years	6/13 (46.2%)	51/67 (76.1%)	**0.029**
6–17 years	1/13 (7.7%)	1/67 (1.5%)	0.734
Adults	76/89 (85.4%)	82/149 (55.0%)	**0.000**
18–60 years	29/76 (38.2%)	25/82 (30.5%)	0.310
> 60 years	47/76 (61.8%)	57/82 (69.5%)	0.310
Inpatients-adults	62/89 (69.7%)	59/149 (39.6%)	**0.000**
Male	39/62 (62.9%)	27/59 (45.8%)	0.058
Age/years	63.9 (15.6)	63.7 (14.0)	0.948
Comorbidities			
Hyperlipidemia	22/62 (35.5%)	14/59 (23.7%)	0.157
Lung diseases	33/62 (53.2%)	33/59 (55.9%)	0.765
Organ transplanted recipients	18/62 (29.0%)	8/59 (13.6%)	**0.038**
Chronic kidney disease	10/62 (16.1%)	13/59 (22.0%)	0.408
Hypertension	31/62 (50.0%)	31/59 (52.5%)	0.780
Cardiovascular diseases	29/62 (46.8%)	29/59 (49.2%)	0.794
Diabetes	20/62 (32.3%)	22/59 (37.3%)	0.561
Malignancy	12/62 (19.4%)	16/59 (27.1%)	0.311
Diagnosis			
Pneumonia	29/62 (46.8%)	21/59 (35.6%)	0.212
Pneumonia in immunocompromised hosts	16/62 (25.8%)	7/59 (11.9%)	**0.051**
Coinfections			
Bacteria	22/62 (35.5%)	14/59 (23.7%)	0.157
Viral	19/62 (30.6%)	11/59 (18.6%)	0.126
Fungus	6/62 (9.7%)	4/59 (6.8%)	0.804
ICU admission	13/62 (21.0%)	10/59 (16.9%)	0.573
Clinical outcome			
Improved	54/62 (87.1%)	51/59 (86.4%)	0.915
Dead	4/62 (6.5%)	3/59 (5.1%)	1.000
Others	4/62 (6.5%)	5/59 (8.5%)	0.938

additional substitutions could collectively contribute to the widespread occurrence of clade A.D.3 [24, 25].

RSV-B sequences investigated in this study belonged to different lineages, namely B.D.4.1.1, B.D.E.1, and B.D.E.2. Notably, a divergent clade, B.D.E.1, exhibited a mutational pattern not detected in pre-pandemic study sequences and was identified as the main cause of the surge in adult infections from P2. Additionally, divergent RSV-B strains characterized by the multiple mutations in mucin-like domain I and II were identified in our strains, some of which have been reported in other countries [8, 26–28]. RSV-B subtypes with notable genetic divergence might have exacerbated its impact due to reduced population immunity against it. The dominant RSV-B lineage emerged very

recently from an evolutionary standpoint, and this recent emergence, coupled with a lack of RSV-B-specific immunity, could have intensified the severity of the 2022–2023 epidemic. Of all, four substitutions, including K256N, I268T, and S275P, were prominent in this novel clade B.D.E.1 and the S100G substitution could have a potential for fitness advantage and global spread demonstrated in an earlier study [29]. Thus, the accumulation of substitutions could influence the immune response and confer a fitness advantage [24, 25]. The mutant viruses should be constructed using reverse genetics techniques to determine the influence of these substitutions on the life cycle, infectivity and pathogenicity.

As for RSV pathogenicity, RSV infection has been demonstrated to be associated with bronchiolitis or pneumonia in children and elderly people, and some cases even progress to severe pneumonia [1, 30–32]. Several studies have reported different conclusions on the infection patterns of different RSV genotypes with the corresponding mutations. In our study, the rate of pneumonia in hospitalized adults, especially in immunocompromised hosts, infected with clade B.D.E.1 is higher than in those infected without clade B.D.E.1 (Table 2). The loss of an N-glycosylation site resulting from T117A substitutions could be responsible for clade B.D.E.1 infection in immunocompromised patients with pneumonia, which was similar to the previous study that suggested the loss of an N-glycosylation site could be responsible for a change in disease severity [33]. The previous studies suggested that the severity of RSV-associated disease was influenced by multiple factors, including host factors, environmental factors, and viral factors [34–37]. Therefore, whether the new clade influences the severity of RSV-associated disease requires further study. However, according to the data on ICU admission and clinical outcome, our results showed no significant difference in clinical outcomes of clade B.D.E.1 infection compared to without clade B.D.E.1 infection. These findings were similar to the results in the previous studies, which suggested that the clinical outcomes of patients infected with different genotypes showed no statistical difference [38]. The host immune response varies among different lineage backgrounds during the evolution of RSV. Further studies are needed to explore the molecular mechanisms of RSV infection in different lineage backgrounds.

There were some limitations in this study. First, this was a single-center study, and all cases in the community and outpatient clinics might not be included. This center is the National Center and has a large number of samples, which, to a certain extent, could represent the majority of the population with respiratory infectious diseases in Beijing. Second, only a partial of sequences rather than the whole sequences were analyzed in this study, while some novel mutations associated with the molecular characterization and disease severity of RSV infection may have mainly depended on the sequences of G protein.

In conclusion, this study revealed an out-of-season epidemic in children and an epidemic outbreak in adults after the end of the Zero-COVID-19 policy in China. The novel imported clade B.D.E.1 was identified as the main cause of the surge in adults. Pneumonia in immunocompromised hosts caused by clade B.D.E.1 was more common compared to other RSV clades. Accumulation of substitutions in clade B.D.E.1 could confer a fitness advantage in vivo. Tracking the molecular evolution and disease outcomes related to RSV is crucial to prevent widespread and severe outbreaks.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The partial RSV G gene sequences identified in this study are available at National Center for Biotechnology Information (BankIt2910027).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.