Clinical, Genomic, and Immunological Characterization of RSV Surge in Sydney, Australia, 2022

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abstract

OBJECTIVES: The 2022 seasonal respiratory syncytial virus (RSV) epidemic in Sydney, Australia saw an unprecedented number of RSV detections. We aimed to characterize genomic and immunologic factors associated with the surge in RSV cases.

METHODS: Whole genome sequences of RSV were generated from 264 RSV-infected infants and linked to case-matched clinical data from the 2022 southern hemisphere RSV season. We then performed an immunologic analysis of baseline RSV-specific humoral immunity in women of childbearing age before and throughout the coronavirus disease 2019 pandemic.

RESULTS: Clinical analysis revealed a high burden of disease across patients of all health backgrounds. More than one-half of RSV-related health care visits by infants resulted in hospitalization, and one-quarter required high-flow respiratory support or a higher level of care. Viral phylogenetic analyses revealed that 2022 Sydney RSV sequences were closely related to viruses that had been circulating globally since 2017, including those detected in recent US outbreaks. Nonsynonymous mutations within the palivizumab and nirsevimab binding sites were detected at low frequencies. There was no difference in baseline RSV-neutralizing antibody titers between 2020 and 2022.

CONCLUSIONS: Collectively, these findings suggest that neither the emergence of a novel RSV genotype nor hypothesized immune debt was associated with the surge of RSV cases and hospitalizations in 2022. Continued genomic and immunologic surveillance is required to further understand the factors driving outbreaks of RSV globally, and to inform guidelines for the rollout and ongoing use of recently developed immunotherapeutics and vaccines.

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Dr Walker conceptualized and designed the study, collected and analyzed the data, and drafted the manuscript; Dr Foster analyzed and interpreted the data and drafted the manuscript; Ms Sevendal, Ms Condylios, and Drs Domazetovska, Williams, Kim, and Stelzer-Braid collected data and supported the interpretation of the data; Dr Kamalakkannan analyzed and interpreted the data; Dr Bartlett and Prof Rawlinson conceptualized and designed the study and supervised data collection and the interpretation of the data; and all authors critically reviewed and revised the manuscript, approved the final manuscript as submitted, and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT: The epidemiology of RSV has been impacted in recent years by public health measures implemented in response to the coronavirus disease 2019 pandemic. In 2022, a surge of RSV cases was detected globally, including in Sydney, Australia.

WHAT THIS STUDY ADDS: The 2022 epidemic in Sydney was driven by preexisting viral lineages, primarily of RSV-B, in contrast to recent US outbreaks driven by RSV-A. At the population level, we did not observe hypothesized immune debt to RSV.

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Respiratory syncytial virus (RSV) causes millions of hospitalizations and up to 118 000 child deaths per year globally, with annual epidemics typically occurring during winter months.[1] However, seasonal patterns of RSV, like many respiratory viruses, have been dramatically impacted by public health measures employed since 2020 in response to the coronavirus disease 2019 (COVID-19) pandemic.[2] The 2020 winter season saw an absence of pediatric RSV cases, followed by unusual off-seasonal summer epidemics occurring in Australia (late 2020) and globally (mid-2021).[3,4] The absence of cases in 2020 also led to a shift in the median age of pediatric cases in subsequent seasons (Supplemental Information), and in 2022, peak RSV case numbers in the state of New South Wales were ~4-fold higher than in 2021 (Fig 1).[5] Comparably large seasonal outbreaks of RSV accompanied by increased numbers of hospitalization were reported in the northern hemisphere during this time.[6–8] Because of recent genomic analyses of 2022 outbreaks in the United States, it was hypothesized that the surge in RSV case numbers resulted from COVID-19 pandemic-related immune debt because the detected genotypes (GA2.3.5 and GB5.0.5.a) have been circulating globally since 2017.[9,10] However, immunologic data are needed to better understand the dynamics of RSV outbreaks, particularly with the imminent availability of novel vaccines and immunotherapeutics.

Until now, the only pharmacological measure for RSV prevention or treatment has been immunoprophylaxis with the monoclonal antibody (mAb) palivizumab.[11] Although available for >20 years, palivizumab use is limited to vulnerable infants in high-resource settings because of cost and the requirement of multiple doses throughout the RSV season. Novel therapeutics and vaccines for RSV prevention are now becoming available for clinical use. A single-dose mAb therapeutic, nirsevimab, has been approved for use by the European Medicines Agency (November 2022) and the United States Food and Drug Administration (FDA, June 2023) as a passive immunization strategy for newborn and infant populations; although it is not yet available in Australia, it is currently under review by the Therapeutic Goods Administration.[12–14]

Vaccines targeting maternal and older adult populations are also undergoing or completing phase 3 clinical trials.[15] These therapeutics are designed to generate host-neutralizing antibodies (NAbs) and provide passive immunization to infants. Current vaccines are directed against one of the antigenic proteins of RSV, the fusion protein, which is required for the virus to fuse with and infect cells.[16] Although the fusion protein is highly conserved, molecular surveillance is required to monitor the emergence and frequency of potential resistance mutations because both naturally occurring and therapy-driven resistance variants have been detected with the use of recently developed mAb and antiviral treatments,[17–20] and the intention is for new RSV therapeutics to be used broadly.

To provide granular data pertaining to unseasonal surges of RSV and evaluate the role mAbs might play in preventing the current morbidity and mortality caused by this virus, whole viral genome sequences of RSV were generated from RSV-infected infants and linked to case-matched clinical data from the 2022 southern hemisphere RSV season. We report factors affecting disease outcomes, characterize local RSV sequences in the context of globally circulating genotypes and newly available

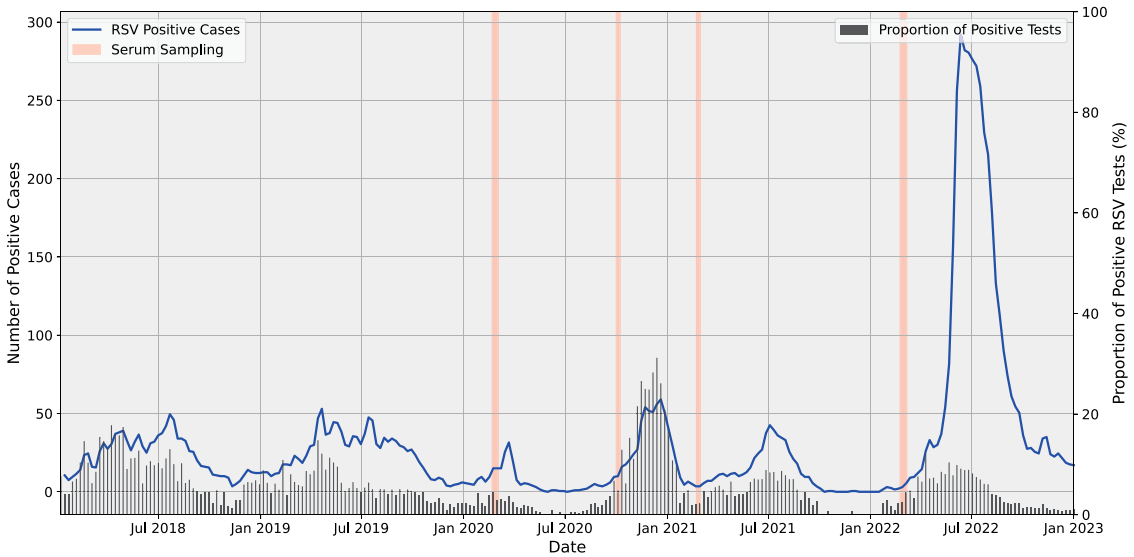

FIGURE 1

The number of weekly RSV detections by polymerase chain reaction (PCR) in the Southeastern Sydney Local Health District over the past 5 years (blue line, left axis) and the proportion of positive RSV test results (black bars, right axis, Supplemental Table 7). The shaded pink area represents serum sampling in women of childbearing age for assessment of RSV-NAbs. Statewide detection data are also available.[5]

RSV therapeutics, and determine baseline RSV-specific humoral immunity in women of childbearing age before and throughout the COVID-19 pandemic.

METHODS

Sample and Clinical Data Collection

Nasopharyngeal swabs discarded after routine diagnostic testing were collected from RSV-positive infants (aged <12 months) who presented with respiratory symptoms to Sydney Children's Hospital Randwick, Australia between May and September 2022. RSV infection was diagnosed by using the Allplex Respiratory Panel 1 (Seegene, South Korea) at the Serology and Virology Division, New South Wales Health Pathology. Specimens (for which there was a sufficient remaining sample) were collected immediately after diagnostic reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and frozen at −80°C until genomic sequencing (Supplemental Information).

Clinical details for identified cases were ascertained via retrospective medical record review to extract data on demographics, comorbidities, level of respiratory support, level of care (outpatient, ward, intensive care), length of hospitalization, and death (Supplemental Information). Treatment with palivizumab did not exclude infants from the study. For analysis, severity was grouped as mild, moderate, or severe. Patients who (1) did not require treatment or received low-flow oxygen were determined to have mild disease, (2) required high-flow oxygen, noninvasive ventilation, or continuous positive airway pressure were determined to have moderate disease, and (3) required mechanical ventilation, or extracorporeal membrane oxygenation or more invasive management, were determined to have severe RSV disease.

Continuous variables were summarized by using median and interquartile range. Comparisons of continuous variables between groups were performed by using 2-tailed Mann-Whitney *U* tests. χ-square tests with Yates' continuity correction were used for the analysis of categorical variables, and Fisher's exact test was used when the expected value of a cell was <5.

RSV Whole-Genome Sequencing and Analysis

Hybridization-capture sequencing of RSV-positive specimens was performed as described in the Supplemental Information. Taxonomic classification of viral reads in all samples was achieved by using CZ ID v7.1, a cloud-based open-source bioinformatics pipeline for metagenomic sequencing data. Viruses other than RSV that were detected at ≥100 reads based on nucleotide alignments were deemed positive and included in coinfection analysis. Deduplicated host-filtered reads were downloaded from CZ ID for in-house variant calling, consensus genome assembly, and phylogenetic analysis, as described in detail in the Supplemental Information.

Analysis of consensus variants focused on nonsynonymous mutations that were supported by a minimum of 20 reads at the consensus level (allele frequency ≥50%). We also investigated minor variants (MV), defined as those variants with an allele frequency of ≥3% and <50% that occurred at genomic positions with a minimum depth of coverage of 200 reads (ie, ≥6 reads supporting the MV out of ≥200 total). Fisher's exact tests were performed to evaluate variants differentially occurring between mild and severe disease groups.

Neutralization Assay

Discarded clinical sera of women 20 to 40 years of age were convenience sampled from the Serology and Virology Division New South Wales Health Pathology Laboratory serology archive before each RSV outbreak occurring between 2020 and 2022 in the Southeastern Sydney Local Health District. Twenty samples were collected during each time period, which included February 2020 to March 2020, October 2020, February 2021 to March 2021, and February 2022 to March 2022 (Fig 1). NAb titers to RSV-A and RSV-B were measured by plaque reduction neutralization test in duplicate, as we have previously described.[21] Ninety-percent inhibitory concentrations (IC_{90}) were calculated by using 4-parameter dose-response curves (GraphPad Prism). Unpaired, 2-sided Student *t* tests were used for statistical comparison between groups.

Ethics Statement

This study was approved by the Sydney Children's Hospitals Network Human Research Ethics Committee (2020/ETH00718).

RESULTS

Clinical Characteristics

Capture-hybridization sequencing was performed on 297 nasopharyngeal swabs collected from 264 RSV-infected infants <12 months of age in Sydney, Australia. There were 104 RSV-A (39.4%) and 160 RSV-B (60.6%) cases.

Clinical data were available for all 264 patients, although severity information was unavailable for 1 patient. Based on the level of respiratory support intervention, 71 (27%) infants were determined to have severe disease. Younger patients (median age 79 days), and those presenting in the month of July 2022 were significantly overrepresented in the severe disease group ($P = .0001$ and $P = .0005$, respectively). Patient sex, comorbidities (including cardiovascular, respiratory, and immunologic), prematurity, RSV subtype, and RSV RT-qPCR cycle threshold value were not significantly associated with disease severity (Table 1 and Supplemental Table 4).

Viral coinfections were detected by using capture-hybridization sequencing in 66 (25.1%) infants (Table 1). In order of frequency, coinfecting viruses included non-

TABLE 1 Characteristics of Infant RSV Cases in Sydney, Australia, 2022

	Total[a]	Mild	Moderate–Severe	*P*
Infants	264	192	71	
Sex, *n*				.1
Male	148 (56.1%)	101 (52.6%)	46 (64.8%)	
Female	116 (43.9%)	91 (47.4%)	25 (35.2%)	
Age at presentation, median d (IQR)	141 (56–245)	175.5 (66.3–266.5)	79 (41–169)	.0001
Subtype, *n*				.87
RSV-A	104 (39.4%)	77 (40.1%)	27 (38.0%)	
RSV-B	160 (60.6%)	115 (59.9%)	44 (62.0%)	
Ct Value, median (IQR)	20.6 (18.0–25.4)	20.5 (18.1–25.8)	21.3 (18.0–25.4)	.72
Sampling (mo), *n*				.0005
May	57 (21.6%)	49 (25.5%)	8 (11.3%)	
June	85 (32.2%)	69 (35.9%)	16 (22.6%)	
July	101 (38.3%)	61 (31.8%)	39 (54.9%)	
August	15 (5.7%)	11 (5.7%)	4 (5.6%)	
September	6 (2.3%)	2 (1.0%)	4 (5.6%)	
Coinfections detected, *n*	66 (25.1%)	53 (27.6%)	13 (18.3%)	.17
Previous health status, *n*				.68
High-risk (preterm or comorbidity)	37 (14.0%)	25 (13.0%)	12 (16.9%)	
Otherwise healthy	227 (86.0%)	167 (87.0%)	59 (83.1%)	

Ct, cycle threshold; IQR, interquartile range.
[a] Severity data incomplete for 1 patient.

SARS coronaviruses, picornaviruses, bocavirus, adenovirus, parainfluenza virus, and influenza A virus (H3N2; Table 2). There was 1 case with 2 co-detections and 1 case with 3 co-detections. Coinfection with any specific virus was not significant ($P = .61$).

Genotyping and Phylogeny

Sequencing was performed in 4 batches of 22, 70, 58, 80, and 67 samples, respectively (Supplemental Table 3). Among specimens from which a complete RSV genome was obtained (>95% coverage), genotyping of the G gene revealed that all RSV-A sequences were GA2.3.5 lineage (ON1-like) and all RSV-B sequences were GB5.05a lineage (BA-like). Phylogenetic analysis with publicly available sequences collected since 2017 revealed that 2022 Sydney RSV (Syd-RSV) sequences generated as part of this study were closely related to RSV viruses circulating globally (Fig 2). All Syd-RSV-A sequences were most closely related to historical US and UK viruses. Syd-RSV-B were clustered with Australian RSV sequences detected during an off-peak RSV outbreak from late 2020 to early 2021 and sequences from the United States (collected from mid- to late 2022), Spain, the United Kingdom, and the Philippines (collected in 2019).

Detection of Antigenic Viral Variants

For variant analysis, we excluded (1) any consensus-level variants (allele frequency ≥50%) occurring at genomic positions with a total read depth of <20 and (2) minor variants (allele frequency ≥3% and <50%) occurring at genomic positions with a total read depth of <200. The distribution and allele frequencies of nonsynonymous mutations across the fusion gene are shown in Fig 3. Antigenic sites Ø (targeted by nirsevimab) and II (targeted by palivizumab) are the target of currently licensed prophylactic mAbs. Antigenic site IV is the target of an mAb undergoing phase 3 clinical trials (clesrovimab), and site V is the target of the discontinued mAb suptavumab.

At the consensus level, for RSV-A, there were 1, 1, and 2 mutations within the antigenic sites II, IV, and V, respectively. For RSV-B, there were 3, 1, 3, and 2 consensus-level mutations detected, within antigenic sites Ø, II, IV, and V, respectively (Fig 3, Supplemental Table 5).

Of note, 1 patient (1%) who was receiving palivizumab prophylaxis had RSV-B with a single-nucleotide polymorphism resulting in K272Q, which has been reported to result in complete loss of palivizumab activity.[22] Within the nirsevimab epitope (site Ø), 1 RSV-B sample (1%) contained the minor variant I64M, which has not been reported elsewhere. The site Ø I206M:Q209R polymorphism detected in the majority of RSV-B samples within our cohort became frequent worldwide between 2015 and 2021, with I206M reported to reduce the neutralization potency of nirsevimab 5-fold.[23,24] Likewise, S211N (detected in 7% of RSV-B genomes) has been extensively characterized and, as a single amino acid change, has no known effect on neutralization by nirsevimab. Across the whole genome, no specific mutations were statistically associated with disease severity.

Assessment of RSV-Specific Antibody Responses

The possibility of waning population immunity to RSV since the beginning of the COVID-19 pandemic was

TABLE 2 Viruses Co-Detected by Capture Hybridization Sequencing (≥100 Reads) in RSV Infection (25.1% of Cases)			
Coinfection Detected, *n* (%)	**Total (*n* = 66)[a]**	**Mild (*n* = 53)**	**Moderate–Severe (*n* = 13)[b]**
Adenovirus	9 (13.6%)	6 (11.3%)	3 (23.1%)
Bocavirus	13 (19.7%)	11 (20.8%)	2 (15.4%)
Influenza A virus (H3N2)	2 (3.0%)	2 (3.8%)	0 (0.0%)
Parainfluenza virus-3	4 (6.1%)	4 (7.5%)	0 (0.0%)
Coronaviruses	25 (37.9%)	19 (35.8%)	6 (46.2%)
229E	8 (12.1%)	6 (11.3%)	2 (15.4%)
OC43	15 (22.7%)	12 (22.6%)	3 (23.1%)
SARS-CoV-2	2 (3.0%)	1 (1.9%)	1 (7.7%)
Picornaviruses	16 (24.2%)	14 (26.4%)	2 (15.4%)
Coxsackievirus	3 (4.5%)	3 (5.7%)	0 (0.0%)
Enterovirus D68	1 (1.5%)	1 (1.9%)	0 (0.0%)
Echovirus	1 (1.5%)	0 (0.0%)	1 (7.7%)
Rhinovirus	11 (16.7%)	10 (18.9%)	1 (7.7%)

[a] 66 cases of coinfection, including $n = 1$ with 2 co-detections and $n = 1$ with 3 co-detections.
[b] No co-detected virus was significantly associated with severity of RSV infection ($P = .61$).

investigated by using serum from women of childbearing age collected at baseline of the past 4 Syd-RSV epidemics (Fig 1). These were tested for NAbs to both RSV-A and RSV-B. The geometric mean of RSV NAb titers did not significantly differ between any of the collection time points for both subtypes (Fig 4). Across all time points, mean neutralizing titers were 318.9 (SD factor 2.66) for RSV-A and 105.1 (SD factor 3.08) for RSV-B (Supplemental Table 6).

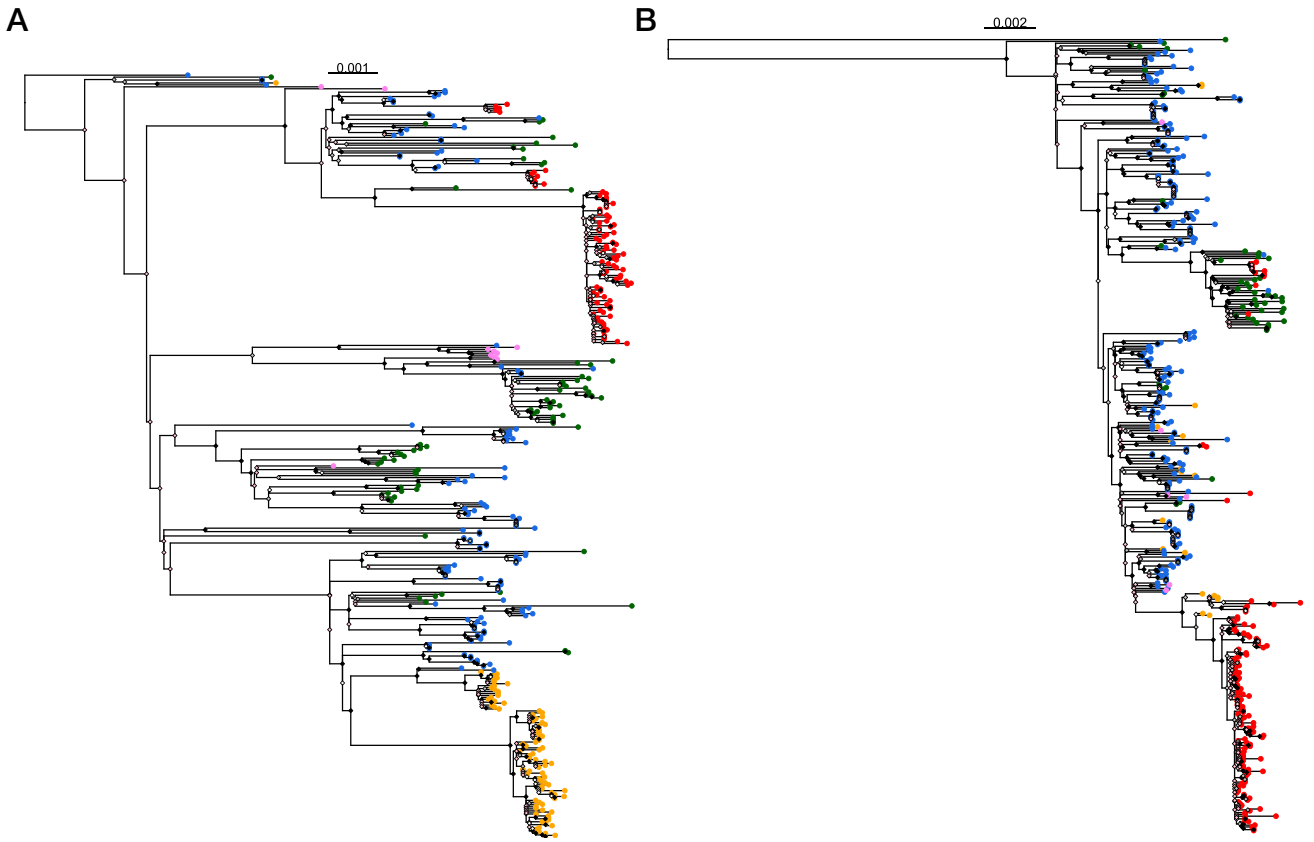

FIGURE 2
Maximum-likelihood phylogenetic trees of complete (A) RSV-A and (B) RSV-B genomes sampled globally from 2017 to 2022. Syd-RSV sequences generated in the current study are represented by red circles at branch tips. Global RSV genomes are represented by orange (other, Australia), violet (Asia), blue (Europe), and green (Americas) circles. The scale bar indicates nucleotide substitutions per site. Diamonds at nodes represent bootstrap support (%), which is a measure of confidence for the phylogenetic estimate at a particular node (black = 100%, gray = 95%–99%, white = 80%–95%, and pink<80%). Files for complete phylogenetic trees are included in the Supplemental Information.

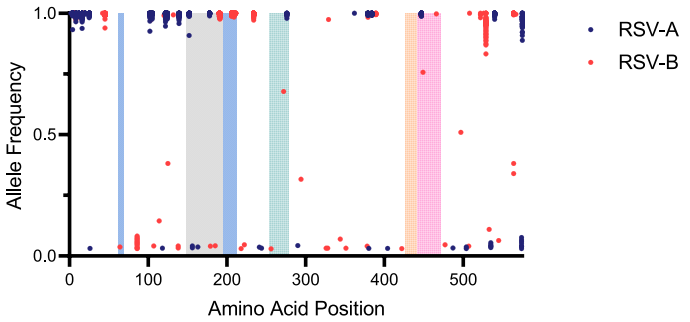

FIGURE 3

Nonsynonymous mutations within the Fusion gene of 118 RSV-A and 179 RSB-B specimens from the 2022 southern hemisphere winter season (Sydney, Australia). Shaded regions represent epitopes of the monoclonal antibodies nirsevimab (blue, antigenic site Ø), suptavimab (gray, antigenic site V), palivizumab (green, antigenic site II), clesrovimab (yellow, antigenic site IV), and 101F (red, antigenic site IV).

DISCUSSION

The significant clinical burden of RSV infection occurs for both immunocompromised and healthy children.[25] In keeping with this notion, our study revealed that in RSV-infected infants <12 months of age, more than one-quarter of hospital visits by otherwise healthy children resulted in admission and receipt of high-flow oxygen support. Although not statistically associated with disease severity, we frequently (25.1%) detected viral coinfections with a hybridization-capture sequencing method utilizing a panel targeting 29 respiratory viruses (>41 000 probes). Although reported rates of viral coinfection with RSV vary widely, partly due to historical limitations in multiplexing capacity and clinical awareness,[26–28] such a high frequency of viral coinfections in our cohort is likely reflective of the coinciding resurgence of other respiratory viruses after recent years of disrupted circulation during the COVID-19 pandemic.[6,29]

The Syd-RSV genotypes detected in our cohort largely reflect those circulating worldwide since 2017, similar to recently reported RSV outbreaks in the United States.[9,10] Evolutionary rates for RSV-A and RSV-B have recently been estimated at 1.48×10^{-3} and 1.92×10^{-3} nucleotide substitutions per site per year, respectively.[30] The branch lengths separating Syd-RSV sequences from historical international samples (collected between 2017 and 2022) are consistent with these published estimates of the evolutionary rates. The close phylogenetic relationship between some RSV-A and -B sequences with recent viruses sampled globally suggests several viral introductions from the northern hemisphere into Sydney in 2022. This follows the dramatically reduced RSV genetic diversity observed in Australia throughout 2020 and 2021 when public health measures related to the COVID-19 pandemic were implemented.[3] Together, our findings reveal

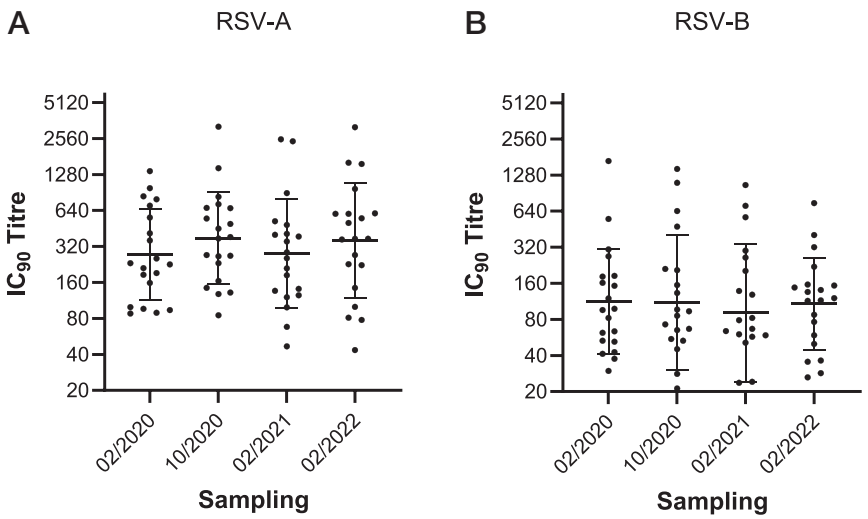

FIGURE 4

(A) Neutralizing RSV-A and (B) RSV-B titers in women of childbearing age since the beginning of the COVID-19 pandemic in Australia. Serum ($n = 80$) collected before the previous 4 local RSV outbreaks (Fig 1) were assayed in duplicate by using a plaque reduction neutralization test, and titers were presented as the reciprocal of the highest serum dilution to inhibit 90% of viral plaque formation in vitro. Three RSV-B samples fell to less than the lower limit of detection (20). Data are presented as geometric mean ± geometric SD. Unpaired, 2-sided Student's *t* tests revealed no statistical significance between any groups.

both the continued endemicity of RSV in Australia during this time period (through clustering of Syd-RSV-B with historical Australian sequences) and a rapid rebound in local genomic diversity after the resumption of international travel.

Repeated maternal viral exposure is considered an important factor in maintaining protective antibody levels in infants. In the absence of circulating RSV infection, NAb titers have been shown to wane rapidly.[31] Indeed, a recent study revealed significant differences in peak–trough NAb titers in 2021 after an absence of RSV cases during the 2020 season.[32] However, although NAb titers vary significantly from peak–trough after an RSV outbreak, we found no difference in the baseline (preoutbreak) NAb titers of serum from women of childbearing age collected before each of the past 4 RSV epidemics occurring in Sydney. These contrasting findings are due to differences in the timing of specimen collection between the 2 studies.[32] Our data indicate that before the 2022 season, antibody-mediated immunity to RSV was, at the population level, no lower than in previous seasons.

Across all seasons, the mean RSV-A neutralizing titers were 3-fold higher than RSV-B neutralizing titers. This may be a consequence of previous immunity to RSV-A in the population stemming from previous epidemics. Whether this contributed to the predominant circulation of RSV-B seen in our study (in contrast to the RSV-A outbreaks observed in the northern hemisphere) requires further investigation. High RSV-A neutralizing titers have previously been associated with protection against all RSV infections, suggesting a level of protective antibody cross-reactivity.[33] The authors of ongoing studies will investigate subtype-specific NAbs in the population after the RSV-B-dominated 2022 season.

An absence of RSV exposure in 2020 resulted in delayed off-season outbreaks seen globally in 2021. Although both the detection of globally established RSV genomes and the high frequency of coinfecting viruses appear to support hypotheses of immune debt as a significant contributor to the size and burden of RSV outbreaks during the 2022 season,[9,10,34] our findings suggest that a more complex interplay of unknown factors, and not waning antibody-mediated immunity, led to the increased RSV case numbers and disease burden observed in 2022 outbreaks. It is also important to note that significant changes in infrastructure, social attitudes, and clinical practice since the COVID-19 pandemic have led to an increased frequency of testing for respiratory infections, making direct comparisons of pre- and post-pandemic RSV detections and rates of positivity difficult. Although the observed surge in cases may be partially attributed to the increased frequency diagnostic of testing, combined clinical and hospital surveillance reports of corresponding RSV outbreaks in the northern hemisphere suggest a genuine rise in RSV transmission and clinical severity in 2022.[4,9,10] In combination with testing data, the consideration of clinical presentations and hospitalization rates is needed in comprehensive epidemiologic studies to provide a holistic understanding of RSV dynamics post-COVID-19.

Because of challenges in developing an infant RSV vaccine, passive immunization through virus neutralization with mAbs is the primary therapeutic option to protect infants against infection in the first 6 months of life, when they are most vulnerable to RSV-induced lower respiratory tract disease. The recently European Medicines Agency- and FDA-approved nirsevimab has high neutralizing activity and enhanced stability and has revealed promising results in protecting infants against severe RSV disease.[35,36] However, prophylaxis with mAbs relies on a high level of conservation with viral epitopes. A primary concern of the enhanced global use of mAbs is the potential selection for, and spread of, mAb-resistant viral variants. We detected the palivizumab resistance mutation K272Q in the sole patient in the cohort receiving palivizumab for RSV. Breakthrough infections by resistant RSV variants are detected in ~5% of patients treated with palivizumab.[22] Although palivizumab-resistant viruses reveal impaired fitness in vitro and only occur at low frequencies within the community,[37] molecular surveillance programs remain important for monitoring therapeutic resistance. For example, in the 2015 to 2017 RSV seasons, a phase 3 clinical trial of mAb suptavumab failed because of the rapid emergence of a high-frequency neutralization-resistant RSV-B variant.[20]

The detection of a single minor I64M variant within antigenic site Ø in our study has not been described elsewhere to date. This amino acid mutation does not alter the polarity from the wild type; however, the characterization of this binding site substitution should be investigated to understand functional changes potentially affecting nirsevimab neutralization. Although conservation within the nirsevimab binding site is high, consensus-level resistance mutations at fusion protein positions 68 and 201 have been found at low frequencies (<1%) in other studies.[23,38] It is unknown whether low-frequency variants detected in RSV molecular surveillance programs will be selected over time with widespread mAb prophylaxis in infants. With the recent approval of the first RSV vaccine for older adult populations by the FDA and UK Medicines and Healthcare products Regulatory Agency,[39,40] ongoing RSV surveillance is also needed to assess selective pressure potentially applied by mAbs and vaccines at the population level and to understand implications of a changing RSV genome for therapeutic efficacy and potential clinical phenotypes.

Limitations of our study include the convenience sampling method evaluating clinical specimens from a single tertiary pediatric hospital, excluding infants with mild RSV illness not seeking medical attention. Although RSV

became a nationally notifiable disease in Australia in 2022, this did not change clinical practice with respect to testing or treatment because all children presenting with respiratory symptoms at Sydney Children's Hospital Randwick are tested for RSV as standard of care. Because of sample availability, we also measured RSV-neutralizing titers in women of childbearing age as a proxy for pediatric samples. Ideally, prospective clinical, virological, and immunologic data from a broad range of infections, including community cases from a larger cohort of infants would enable a more comprehensive analysis of variables related to RSV. However, our data are broadly reflective of the local and international reports of increased RSV detections and burden during the COVID-19 pandemic.[5,7]

CONCLUSIONS

Our findings suggest that a complex interplay of factors, beyond a simplistic reduction in population antibody-mediated immunity, contributed to the variability of seasonal RSV epidemics. Continued genomic and immunologic surveillance programs are required to understand these factors and devise optimal strategies for managing RSV outbreaks, particularly in the context of the evolving landscape of immunotherapeutics. Such studies can inform the future design and use of vaccines, particularly mRNA redesign postvaccine utilization.

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ABBREVIATIONS

COVID-19: coronavirus disease 2019
ECMO: extracorporeal membrane oxygenation
FDA: US Food and Drug Administration
IC_{90}: 90% inhibitory concentrations
mAb: monoclonal antibody
MV: minor variants
NAb: neutralizing antibody
RSV: respiratory syncytial virus
RT-qPCR expansion: reverse transcription-quantitative polymerase chain reaction
Syd-RSV: Sydney respiratory syncytial virus

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DATA SHARING: Nonhost sequencing reads generated by this study are publicly available on the Sequence Read Archive (BioProject ID: PRJNA1037681).

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