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The changing etiology of NICU-Admitted neonatal viral pneumonia: A multiplex RT-PCR era snapshot

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ABSTRACT

Objective: Viral pathogens are an important cause of lower respiratory tract infection (LRTI) leading to hospitalization in neonates and young infants. This study evaluated the etiologic distribution, clinical course, and management of multiplex RT-PCR–confirmed LRTI among neonatal intensive care unit (NICU) admissions.

Materials and Methods: Between September 2022 and November 2023, neonates admitted to the NICU with LRTI and a positive respiratory viral panel (multiplex RT-PCR) were retrospectively analyzed (n=44). Disease severity at admission was assessed using the Downes score. Demographics, seasonality, respiratory support, treatments, and outcomes were recorded. Correlations between Downes score, oxygen requirement (FiO_2), and length of stay were examined.

Results: Median age at admission was 17 days (range: 1–45). Admissions peaked in January (29.5%) and November (18.1%). The most common presenting findings were respiratory distress/tachypnea, cough, and feeding difficulties. Median Downes score was 2 (range: 1–8) and median FiO_2 requirement was 31% (range: 25–45%). Respiratory support was required in 32 infants: mechanical ventilation in 11.4% (n=5), non-invasive ventilation in 22.7% (n=10), and supplemental oxygen in 38.6% (n=17). The most common agents were SARS-CoV-2 (n=22; 50%) and respiratory syncytial virus (RSV) (n=14; 31.8%). Downes score correlated positively with FiO_2 (r=0.39, p=0.008) and length of hospital stay (r=0.42, p=0.004). Oseltamivir was administered to three infants with influenza. Median length of stay was 6 days (range: 2–56) and no mortality occurred.

Conclusion: In this multiplex RT-PCR era snapshot, SARS-CoV-2 and RSV predominated among NICU-admitted neonates and young infants with viral LRTI. Management was primarily supportive, and admission Downes score may help anticipate oxygen requirement and hospitalization duration.

Keywords: Neonate, NICU, respiratory syncytial virus, SARS-CoV-2, viral pneumonia

Introduction

Advances in perinatal and neonatal care have substantially improved survival for preterm and critically ill newborns; however, respiratory infections continue to impose a major clinical burden in early life. Viral respiratory diseases account for a large share of pediatric respiratory hospitalizations, and their impact is particularly relevant for the youngest and most vulnerable patients, including those requiring intensive care (1).

In hospitalized children, molecular studies consistently show that viral pathogens account for a substantial proportion of acute respiratory infections, supporting virus-focused diagnostics and management strategies (2).

Although respiratory viral infections (RVIs) are considered relatively infrequent during birth hospitalization, they can lead to significant short- and long-term morbidity in both term and preterm neonates. In the neonatal intensive care unit (NICU), RVIs have been associated with a more severe disease course, prolonged hospitalization, unnecessary antimicrobial exposure, and nosocomial outbreaks, partly because clinical presentations often overlap with bacterial infections (3). The COVID-19 pandemic further altered respiratory virus circulation patterns, and NICU surveillance

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studies have shown that the epidemiology of viral detections during admission can shift across pre- and post-pandemic periods (4).

In this context, accurate etiologic identification is central to both clinical management and infection prevention. Modern molecular assays, particularly multiplex RT-PCR panels, enable timely detection of a broad range of respiratory viruses and support targeted cohorting and antimicrobial stewardship in NICU settings (3,4). Moreover, different viruses may be associated with distinct clinical and laboratory profiles, reinforcing the value of virologic confirmation when respiratory disease is suspected (5).

Therefore, this study aimed to evaluate the etiologic distribution, clinical course, and management approaches among neonates and young infants admitted to our NICU with multiplex RT-PCR-confirmed viral lower respiratory tract infection.

Materials and Methods

Study design, setting, and participants

This retrospective observational study evaluated term babies between 0-28 days and preterm babies with a postmenstrual age up to 44 weeks who were admitted to our NICU after presenting to the emergency department with respiratory distress between September 2022 and November 2023. During the study period, a respiratory viral panel using multiplex RT-PCR was performed in 72 patients with suspected viral lower respiratory tract infection (LRTI). Infants with a positive multiplex RT-PCR result (n=44) were included in the final analysis. Infants with severe congenital malformations were excluded.

Data collection

Clinical and demographic variables were extracted from the hospital electronic medical record system, including gestational age, mode of delivery, postnatal age at admission, sex, birth weight, number of siblings, number of household members, history of LRTI within the household, presenting symptoms, type of respiratory support at admission, oxygen requirement (FiO_2), chest radiography findings, laboratory results, administered treatments (including antiviral/antibacterial therapy), and length of hospital stay.

Severity assessment

On the day of admission, respiratory distress severity was assessed using the Downes score, a bedside clinical scoring system originally described for grading neonatal respiratory distress and correlating with physiologic derangements (6). On the day of initial presentation, the Downes score, a comprehensive scoring system applicable to neonates of any gestational age, was utilized to assess the severity of respiratory distress and to facilitate objective decision-making regarding the initiation of treatment (Table I) (7,8).

Table I: Downes scoring system

Downes Score	0	1	2
Respiratory Rate	<60/min	60–80/min	>80/min or apnea
Retractions	Absent	Mild	Severe
Cyanosis	Absent	In room air	>40% FiO_2
Air Entry	Good	Decreased	Barely audible
Grunting	Absent	Audible with stethoscope	Audible without stethoscope

≥4 points: *Clinical respiratory distress,* ***>7 points:*** *Respiratory failure*

Clinical management and treatment decisions

All patients received supportive management as clinically indicated, including supplemental oxygen, invasive mechanical ventilation and/or non-invasive ventilation, fluid and electrolyte support, inotropic therapy when required, and enteral/parenteral nutrition. Antibacterial and/or antiviral therapy was initiated according to the attending neonatologist's decision. In suspected influenza cases, antiviral treatment (oseltamivir) was considered/used per clinical judgment, consistent with prior NICU literature addressing influenza management considerations in newborns (9).

Criteria for initiating antibacterial therapy

Antibacterial therapy was initiated when bacterial pneumonia could not be excluded, based on one or more of the following: high fever; progressively increasing oxygen requirement to maintain target oxygen saturation (90–92% in preterm infants and 94–96% in term infants); worsening respiratory distress despite treatment; clinical deterioration; radiographic evidence suggestive of infiltration; positive blood culture and/or tracheal aspirate culture; leukocytosis (>15.000/mm (3)); and elevated C-reactive protein (CRP >5 mg/L).

Statistical analysis

Statistical analysis were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Chicago, IL, USA). Descriptive statistics were used throughout. Continuous variables were summarized as mean and standard deviation (SD) for approximately normally distributed data, and as median (min–max) for non-normally distributed data. Categorical variables were presented as counts (n) and percentages (%). Associations between the Downes score and (i) oxygen requirement (FiO_2) and (ii) length of hospital stay were assessed using Spearman's rank correlation coefficient. All tests were two-tailed, and a p value <0.050 was considered statistically significant.

Results

All patients were previously healthy newborns without known chronic comorbidities. The monthly distribution of admissions is shown in Figure 1, with the highest frequency in January (29.5%) and November (18.1%).

Baseline characteristics

Sociodemographic and clinical characteristics are summarized in Table II. Median maternal age was 26.5 (range: 17-32) years. Median birth weight was 2980 g (range: 1680-4500) and median gestational age was 36.45 weeks (range: 34-41). Delivery by cesarean section occurred in 61.4% (n=27), and 59.1% (n=26) of the infants were male. Median 5-minute APGAR score was 8.0 (range: 3–9). Median postnatal age at admission was 17 days (range: 1–45). No infant had a history of prolonged rupture of membranes (>18 h).

Table II: Sociodemographic and clinical characteristics of all patients

Variables	Values
Maternal age (years)*	26.5 (17-32)
Gestational age (weeks)*	36.45 (34-41)
Mode of delivery (cesarean section)†	27 (61.4)
Birth weight (g)*	2980 (1680-4500)
Gender (male)†	26 (59.1)
APGAR score (5th min)*	8.0 (3–9)
Age at admission (days)*	17 (1–45)
Downes score*	2.0 (1–8)
Respiratory support†	32 (72.7)
Mechanical ventilation (MV)	5 (11.4)
Non-invasive ventilation (NIV)	10 (22.7)
Supplemental oxygen	17 (38.6)
Duration of MV (days)*	0 (0-20)
Duration of NIV (days)*	2.5 (0-16)
Length of hospital stay (days)*	6 (2–56)

:** *median (min-max),* **†:** *n(%),* ***MV: *mechanical ventilation,* ***NIV:*** *non-invasive ventilation*

Clinical presentation and exposure history

At admission, 59.1% (n=26) of infants were afebrile. The most common presenting symptoms were respiratory distress/tachypnea (n=28; 63.6%), feeding difficulty (n=24; 54.5%), and cough (n=22; 50%). Median Downes score at admission was 2.0 (range: 1–8). The household history of LRTI exposure was reported in 45.5% (n=20). Having a sibling attending daycare/school was present in 61.3% (n=27), and 61.3% (n=27) lived in households with >3 persons.

Virologic findings and secondary bacterial pathogens

According to multiplex RT-PCR, the most frequently detected viruses were SARS-CoV-2 (n=22; 50%) and RSV (n=14; 31.8%) (Figure 2). Secondary bacterial pathogens were identified as Streptococcus pneumoniae (n=3; 6.8%) and Haemophilus influenzae (n=1; 2.3%).

Severity, respiratory support, and outcomes

A Downes score ≥4 was observed in 15 infants (34%), and >7 in 2 infants (4.5%). Downes score showed a positive correlation with FiO_2 requirement (r=0.39, p=0.008) and length of hospital stay (r=0.42, p=0.004) (Figures 3–4). Laboratory values at admission were: median CRP 1 mg/L (range: 0-45) , WBC 8440/mm^3 (range: 5020-12340), lymphocytes 4365/mm^3 (range: 1310–6160), MPV 10.25±2.06 fL, absolute neutrophil count 2345 (range: 1170-6570) and neutrophil-to-lymphocyte ratio 0.64 (0.27-2.37).

Chest radiography showed ground-glass opacities in 68.2% (n=30) and paracardiac infiltrates in 31.8% (n=14). Median FiO_2 on day 1 was 31% (range: 25–45). Overall, 32 infants required respiratory support: mechanical ventilation 11.4% (n=5), non-invasive ventilation 22.7% (n=10), and supplemental oxygen 38.6% (n=17). Intravenous fluids/electrolytes and nutritional support were provided when indicated. Oseltamivir was administered in three infants with influenza. Antibiotics (ampicillin+gentamicin or cefotaxime) were initiated in 32 infants due to suspected bacterial pneumonia and discontinued after viral etiology was identified.

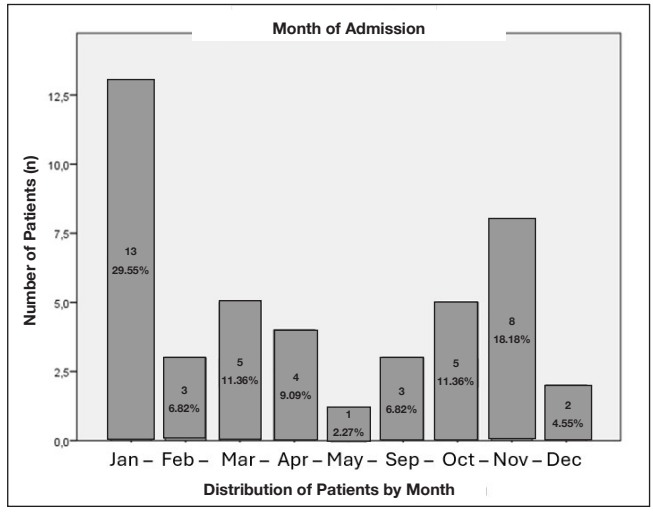


Figure 1: Monthly distribution of patient admissions

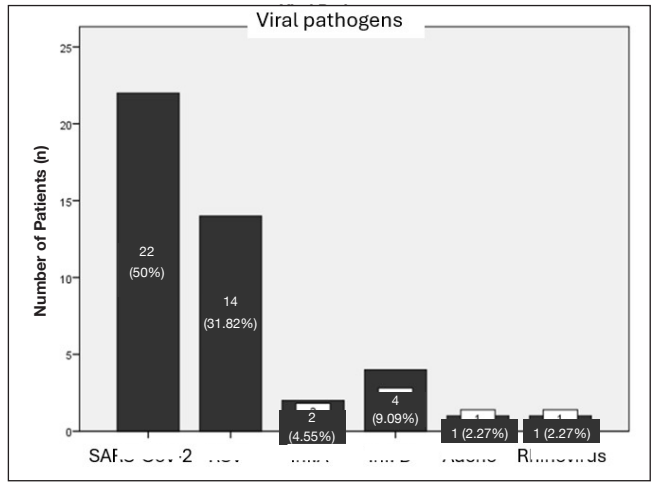


Figure 2: Distribution of viral pathogens

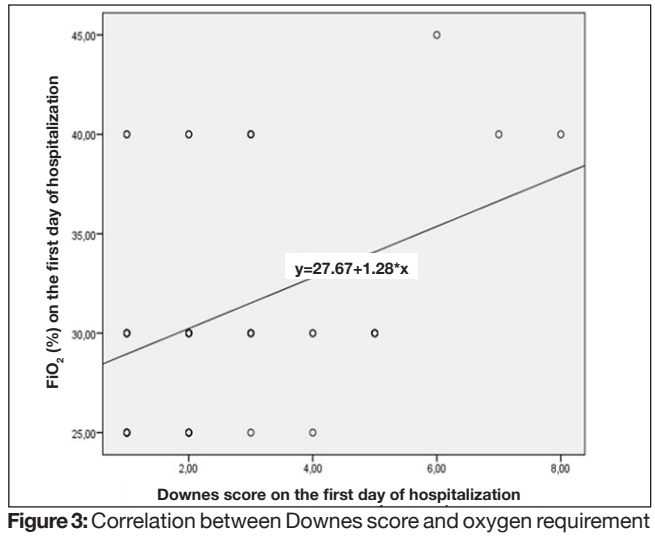


Figure 3: Correlation between Downes score and oxygen requirement (FiO_2)

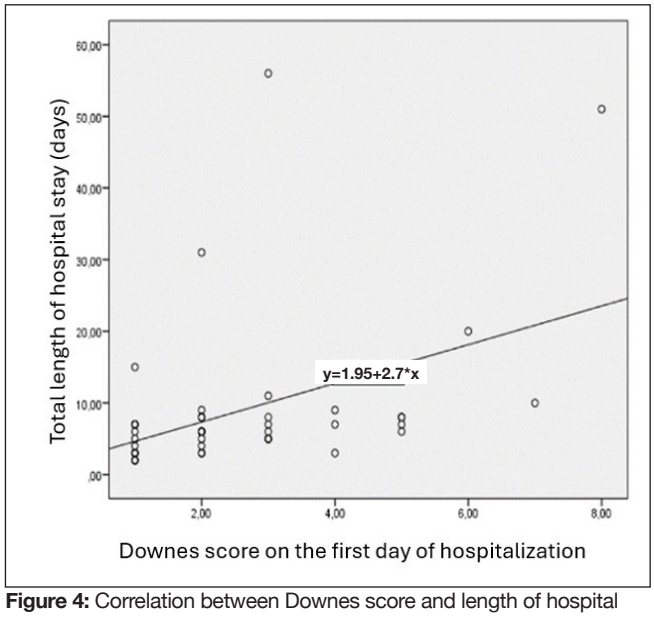


Figure 4: Correlation between Downes score and length of hospital stay

Median length of stay was 6 days (range: 2–56). No mortality occurred, and all infants were discharged with clinical and laboratory recovery.

Discussion

In this multiplex RT-PCR–era snapshot of NICU-admitted neonates and young infants with viral LRTI, admissions clustered in the colder months, with detections beginning in early autumn and peaking in winter. The rationale for etiologic confirmation with molecular testing and its relevance to infection prevention and antimicrobial stewardship in NICU settings have been emphasized in prior NICU-focused respiratory virus surveillance and review studies (3,4). Seasonality of respiratory viral infections is well described and reflects interactions between environmental conditions, host susceptibility, and viral transmission dynamics (10). NICU surveillance studies have shown that the burden and epidemiology of viral respiratory infections can change over time and shift under the influence of broader public health measures (4). Post-pandemic reports from several settings suggest altered timing and intensity of RSV activity and other respiratory viruses, and hospital-based analyses of recent respiratory seasons demonstrate that circulating viruses and clinical outcomes vary across years (11-13). Etiologic profiles of pediatric acute respiratory infection hospitalizations have also been reported to change after relaxation of COVID-19 non-pharmacological interventions, contributing to discussion around "immunity debt" and the need to sustain routine prevention strategies (14,15).

The most frequently detected pathogens in our cohort were SARS-CoV-2 and RSV. Surveillance data indicate that dominant etiologic agents can vary by population, testing practices, and pandemic/post-pandemic dynamics (4,13). Turkish NICU series evaluating neonatal viral LRTI admissions similarly highlight the persistent burden of viral respiratory disease and the common need for NICU-level monitoring and respiratory support (16,17). With respect to SARS-CoV-2, available evidence in maternal–neonatal outcome literature suggests a low likelihood of vertical transmission, while postnatal acquisition remains plausible, particularly via household exposure and close contact (18). In our cohort, household exposure markers (daycare/school-aged siblings and larger households) were common, supporting continued counseling for families of newborns and practical unit-level measures during periods of high community transmission.

RSV remains a major pathogen in neonatal and pediatric respiratory disease (19). In NICU settings, RSV transmission is facilitated by close contact and contaminated surfaces, and RSV has been detected within neonatal intensive care environments, supporting the need for rigorous infection-control practices (3,20). Globally, RSV contributes substantially to the burden of acute lower respiratory infection and mortality in young children, and severe disease is more likely in vulnerable populations and in those with important comorbidities, including HIV infection (21,22). In neonates requiring NICU admission, RSV pneumonia may present with significant respiratory distress, yet outcomes can be favorable with appropriate supportive care and monitoring, consistent with our zero-mortality findings (8).

Accurate etiologic identification is central to both clinical management and infection prevention. Routine clinical, laboratory, and radiographic findings may not reliably distinguish viral from bacterial disease early in the course, as emphasized in pediatric and NICU literature (3,23). It is important to acknowledge that the high sensitivity of multiplex molecular panels allows for the detection of organisms that may be part of the normal upper respiratory flora or represent colonization rather than active infection. Bacterial targets frequently included in these panels, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, are known to colonize the nasopharynx in young children. Consequently, the detection of bacterial nucleic acids does not always indicate a causal role in the acute illness. In the present study, we interpreted positive bacterial results in conjunction with clinical presentation, radiological findings, and inflammatory markers to distinguish true pathogens from bystanders, as recommended in recent literature (24). In our cohort, empiric antibiotics were commonly initiated due to this diagnostic uncertainty; similarly, prospective NICU data show that viral infections may be present among neonates evaluated for suspected late-onset bacterial sepsis, reinforcing the value of virologic testing in stewardship-focused care (25). Multiplex RT-PCR respiratory panels and other viral molecular panels can support patient cohorting/isolation and earlier discontinuation of avoidable antibiotics when bacterial infection is not supported (3,4,26). In addition, differential laboratory patterns across respiratory viruses have been reported; such parameters may complement clinical assessment but are insufficient alone for etiologic assignment (5). Our observations regarding risk factors align with local data reported by Çelik et al. (27), who identified the presence of siblings in the household (72%), family history of viral infection, lack of breast milk intake, Bronchopulmonary Dysplasia (BPD), and siblings attending school as major risk factors. Although RSV remains the predominant etiology of neonatal acute lower respiratory tract infections, it is crucial

to emphasize that the clinical impact of rare pathogens and co-infections must not be underestimated. Reducing unnecessary antibiotic exposure is particularly relevant because respiratory illness after NICU discharge remains an important cause of rehospitalization among preterm infants, and long-term respiratory vulnerability has also been described among survivors of early neonatal respiratory disease (28,29).

Most infants in our cohort required some form of respiratory support, underscoring that viral LRTIs can cause clinically meaningful respiratory compromise even in previously healthy neonates. National guidance emphasizes structured assessment and stepwise supportive management of respiratory distress in term neonates (7). Escalation to non-invasive ventilation or mechanical ventilation introduces additional risks, and consistent adherence to strategies for preventing ventilator-associated complications, including ventilator-associated pneumonia, is essential in NICU practice (30). The male predominance observed in our cohort is also consistent with broader neonatology references noting sex-related differences in susceptibility and clinical presentation in newborn infections (31).

We also observed that the admission Downes score correlated positively with oxygen requirement (FiO_2) and length of hospital stay. The Downes (Vidyasagar) score was originally described as a bedside clinical scoring system for grading neonatal respiratory distress, and its incorporation into routine pathways may assist early risk stratification and harmonize escalation decisions across providers (6,7).

Influenza was uncommon in our cohort; nevertheless, it remains clinically important because outbreaks in neonatal units have been described and targeted antiviral therapy may be considered in selected cases (9). Reviews of neonatal viral infections emphasize that supportive care is the cornerstone of management for most viral pathogens, while pathogen-specific therapy is limited to select viruses (e.g., influenza), and unnecessary antibiotics should be avoided in the absence of bacterial evidence (32).

Limitations

Limitations of this study include its retrospective single-center design, modest sample size, and selection of infants tested for suspected viral LRTI, which may limit generalizability. Multiplex RT-PCR turnaround time may have influenced antibiotic exposure and length of stay. The study was not designed to quantify viral–bacterial coinfection rates robustly or to compare outcomes with a PCR-negative control group.

Conclusion

In conclusion, NICU-admitted neonates and young infants with multiplex RT-PCR–confirmed viral LRTI showed clear winter predominance, with SARS-CoV-2 and RSV as the leading detected pathogens. These findings support continued emphasis on timely molecular diagnostics, infection prevention, and primarily supportive management, while structured bedside severity scoring may assist early clinical decision-making and stewardship-focused care (3,4,6,7,32).

Ethics committee approval

This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Ankara Etlik City Hospital Ethics Committee (03.09.2025, reference number: 2025-434).

Contribution of the authors

Conceptualization/Planning: AC, DD, HA, AO; Analysis/Interpretation: AC, DD, HA, SA; Data Acquisition: AC, DD, HA, AO, EO, SA, NYY, NF, FK; Writing: AC, DD, HA; Review and Editing: AC, DD, HA; Approval: AC, DD, HA

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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