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Original Article

Prevalence and associated factors of pneumococcal carriage among children with respiratory viral infections in Türkiye

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Abstract

Introduction: The interaction between nasopharyngeal pneumococcal carriage (NPC) and respiratory viral infections (RVI) is poorly understood. This study aimed to investigate the prevalence and epidemiology of NPC, and its correlation with RVI.

Methodology: This retrospective study included patients aged < 18 years who presented with RVI symptoms between 1 February and 30 June 2023. Naso-oropharyngeal swabs were tested using quantitative polymerase chain reaction (PCR) for *Streptococcus pneumoniae* and respiratory viruses. Patients were grouped by NPC status and compared for viral detection, and clinical and demographic characteristics.

Results: NPC was detected in 15.7% (n = 1,185) of the 7,522 samples analyzed. The mean age was 56.5 ± 49.3 months, being significantly lower in NPC-positive patients ($p < 0.001$). Most (82%) were fully vaccinated against pneumococcus; 16% had underlying conditions. The most frequent viruses were adenovirus (17.2%), influenza B (9.0%), severe acute respiratory syndrome coronavirus 2 (5.3%), respiratory syncytial virus (4.9%), and influenza A (2.8%). Adenovirus (19.7%) and influenza B (11.0%) were significantly more frequent in NPC-positive patients than in NPC-negative patients ($p < 0.001$ for both). Overall, 20.5% were hospitalized, and 2.4% required intensive care. Intensive care admission was higher among NPC-positive patients, whereas mortality did not differ.

Conclusions: NPC was epidemiologically associated with the detection of certain respiratory viruses, particularly adenovirus and influenza B. Given the retrospective design and lack of pneumococcal serotype data, these findings should be interpreted as associations rather than evidence of causality. Further prospective studies are warranted to clarify the bacterial–viral interactions and their clinical relevance.

Key words: carriage; *Streptococcus pneumoniae*; respiratory; viruses; coinfection; pediatrics.

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Introduction

Streptococcus pneumoniae is a leading cause of invasive bacterial disease and a major contributor to morbidity and mortality in children [1]. The microorganism can be carried asymptomatically, and the main reservoir site for its carriage is the nasopharynx. Nasopharyngeal pneumococcal carriage (NPC) is a prerequisite for the development of pneumococcal diseases and plays a key role in human-to-human transmission [2,3]. However, NPC does not always lead to clinical disease, and its determinants are diverse [2,4]. Despite the nasopharynx being the main site of pneumococcal carriage, *S. pneumoniae* is not a leading bacterial cause of tonsillopharyngitis [2,3]. The main carriers of this pathogen are infants, especially children < 5 years of age [3].

The prevalence of NPC is influenced by multiple factors [3]. Since the global implementation of pneumococcal conjugate vaccines (PCVs), there has been a marked decline in invasive pneumococcal diseases (IPDs) and hospitalizations due to pneumonia of all causes [5]. Furthermore, vaccination has led to a decrease in the carriage of vaccine-included serotypes, along with a shift toward non-vaccine serotypes [6]. While numerous studies have examined NPC in healthy children, data on carriage patterns in ill children with infections are lacking [2,4].

Respiratory tract infections (RTIs) are most commonly caused by viruses [7]. With advancements in diagnostic methods, co-infections involving both viruses and bacteria have been increasingly reported [8,9]. It is well established that viral infections predispose individuals to secondary bacterial infections [10]. Conversely, bacterial colonization may also play a role in the development or severity of RTIs [11,12]. Some viral infections are associated with an increased bacterial load in the nasopharynx [13–15].

The interaction between bacterial colonization and

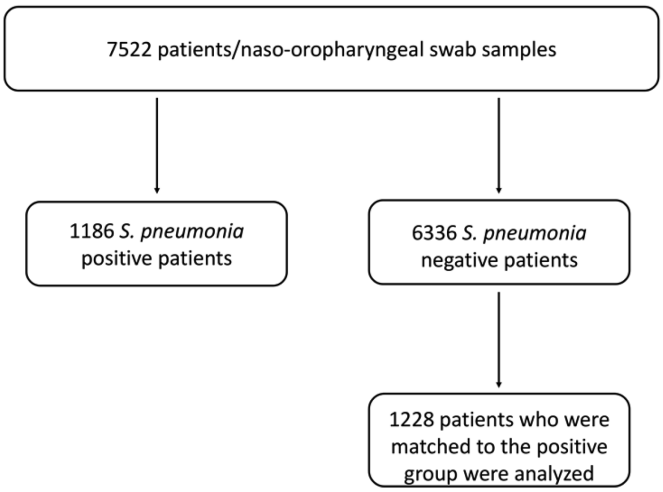
viral co-infection is complex. Although both direct and indirect mechanisms are believed to be involved, the exact nature of this interplay remains poorly understood [16–18]. The primary aim of this study was to investigate NPC and co-infection dynamics during viral respiratory infections in children and to contribute novel insights to the existing literature on this subject.

Methodology

This study was conducted at Ankara Bilkent City Hospital, a university-government partnership hospital providing tertiary healthcare services in the capital of Türkiye. The hospital has a total bed capacity of 4,050, including 622 pediatric beds. Children aged < 18 years, who presented to the pediatric wards with any symptoms of respiratory tract infection (RTI) between 1 January 2023 and 30 June 2023, and who underwent naso-oropharyngeal swab sampling, were included in the study. Demographic data, pneumococcal vaccination status, presence of underlying disease, antibiotic use within the last month, infection category, hospitalization status, intensive care unit (ICU) requirement, naso-oropharyngeal sampling results, and mortality outcomes were obtained from the hospital's electronic medical record system. Pneumococcal vaccination status was verified using the national personal health record system "e-Nabız," provided by the Republic of Türkiye Ministry of Health.

Vaccination status was categorized into three groups: patients with no prior doses of any PCV (PCV7 or PCV13) were considered "unvaccinated", those who had received some doses but were not fully vaccinated according to their age and the national immunization schedule were classified as "incompletely vaccinated", and patients who had received all age-appropriate doses as recommended by the national immunization schedule were considered "fully vaccinated".

Figure 1. Distribution of *Streptococcus pneumoniae* positive and negative groups.



The final infection category was determined based on clinical, laboratory, and radiological evaluations; and classified as upper RTI (tonsillopharyngitis and/or rhinosinusitis), acute otitis media (AOM), conjunctivitis, lower RTI, acute gastroenteritis, and other infections (e.g., sepsis, meningitis).

Sample collection and laboratory testing

Naso-oropharyngeal samples were collected by trained healthcare professionals using the standardized procedures recommended by the World Health Organization (WHO) Working Group [19]. Although nasopharynx is the primary ecological niche for pneumococcal carriage and isolated nasopharyngeal sampling is generally recommended for pneumococcal detection [2], the diagnostic kit used in this study allowed simultaneous viral and bacterial co-detection. Therefore, specimen collection was performed using combined nasopharyngeal and oropharyngeal swabs, as recommended by the diagnostic kit and consistent with routine clinical practice for respiratory pathogen surveillance.

After collection, the swabs were placed in a transport medium (vNAT, Bioeksen, İstanbul, Türkiye) and transported to the laboratory within two hours under appropriate conditions. Respiratory pathogens were identified using the Bio-Speedy® Respiratory RT-qPCR MX-24 panel (Bioeksen, İstanbul, Türkiye), which detects 17 viruses (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], adenovirus [AdV], bocavirus, coronavirus 229E/HKU1/NL63/OC43, human metapneumovirus, influenza virus A, influenza virus B, parainfluenza virus [PIV] 1/2/3/4, respiratory syncytial virus [RSV] A/B, enterovirus/rhinovirus, parechovirus) and 6 bacteria (*Bordetella pertussis*, *Chlamydophila pneumophila*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Streptococcus pneumonia*, and *Haemophilus influenzae*). The samples were processed using a CFX96 Touch real-time polymerase chain reaction (PCR) system (Bio-Rad Laboratories, Mannheim, Germany).

The quantitative reverse transcription PCR (RT-qPCR) assay provided qualitative detection of *S. pneumoniae* DNA. Cycle threshold values or quantitative bacterial density measurements were not available; therefore, pneumococcal positivity was considered with caution in the interpretation of the results and was not used as an indicator of colonization

density or invasive potential.

Statistical analysis

Statistical analyses were performed using SPSS version 25.0 (IBM SPSS Statistics, Chicago, IL, USA). Descriptive statistics were presented as numbers and percentages for categorical variables; and as mean ± standard deviation (SD), median, interquartile range (IQR), and minimum–maximum for continuous variables. For comparison of continuous variables, the Mann–Whitney U test was used for two independent groups, following assessment of normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Categorical variables were analyzed using the χ^2 and Fisher's exact tests. The Bonferroni correction was applied in the post-hoc analysis of categorical variables. In order to identify factors associated with pneumococcal carriage, variables with $p < 0.05$ in univariate analyses were included in a multivariate analysis using a binary logistic regression model. Age and pneumococcal vaccination status were strongly correlated; therefore, age was not included in the multivariate logistic regression model to avoid multicollinearity. Despite multivariable adjustment, residual confounding related to age, disease severity, healthcare-seeking behavior, and testing indications could not be fully excluded. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for the analysis. Statistical significance was set at $p < 0.05$.

Results

A total of 7,522 naso-oropharyngeal samples were collected from 7,522 patients during the study period. *S. pneumoniae* was detected in 1,186 (15.7%) samples. In addition, 1,228 patient records were selected from among the samples that tested negative for *S. pneumoniae* using a stratified random sampling method and matched with the positive group in terms of number and gender distribution (Figure 1).

Demographic characteristics and clinical parameters

Demographic data, pneumococcal vaccination status, and history of antibiotic use before admission were evaluated for all patients and compared between the *S. pneumoniae*-positive and *S. pneumoniae*-negative groups (Table 1). The mean age of the study population was 56.5 ± 49.3 months (range: 0–215 months), and the mean age was significantly lower in

Table 1. Demographic data, pneumococcal vaccination status, history of antibiotic usage before admission, infection-related parameters, hospitalization and intensive care need, and mortality.

Characteristics	Total n = 2414	*S. pneumonia* positive n = 1186	*S. pneumonia* negative n = 1228	*p* value
Age in months, mean ± SD	56.51 ± 49.27	49.12 ± 42.29	63.63 ± 54.25	< 0.001
Age groups, n (%)				
< 12 months	452 (18.7)	283 (23.9)	169 (13.8)	
12–23 months	334 (13.8)	138 (11.6)	196 (16)	< 0.001
24–59 months	657 (27.2)	340 (28.7)	317 (25.8)	
> 60 months	971 (40.2)	425 (35.8)	546 (44.5)	
Gender (male), n (%)	1460 (60.5)	714 (60.2)	746 (60.7)	0.784
Pneumococcal vaccination status, n (%)				
Unvaccinated	91 (3.8)	40 (3.4)	51 (4.2)	
Incompletely vaccinated	383 (15.9)	244 (20.6)	139 (11.3)	< 0.001
Fully vaccinated	1939 (80.4)	902 (76.1)	1037 (80.4)	
Underlying disease, n (%)				
Neurological	110 (4.6)	48 (4.1)	62 (5)	
Metabolic	36 (1.5)	12 (1)	24 (2)	
Pulmonological	51 (2.1)	28 (2.4)	23 (1.9)	
Immunological	80 (3.3)	36 (3)	44 (3.6)	
Cardiological	31 (1.3)	19 (1.6)	12 (1)	< 0.001
Hemato-oncological	76 (3.1)	28 (2.4)	48 (3.9)	
Endocrinological	22 (0.9)	10 (0.8)	12 (1)	
Genetic	18 (0.7)	10 (0.8)	8 (0.7)	
Nephrological	31 (1.3)	4 (0.3)	27 (2.2)	
Antibiotic usage in the last month, n (%)	423 (17.5)	210 (17.7)	213 (17.3)	0.815
Infection category, n (%)				
Upper RTI	1647 (68.2)	770 (64.9)	877 (71.4)	
Acute otitis media	71 (2.9)	37 (3.1)	34 (2.8)	
Conjunctivitis	54 (2.2)	23 (1.9)	31 (2.5)	0.009
Lower RTI	495 (20.5)	276 (23.3)	219 (17.8)	
Acute gastroenteritis	67 (2.8)	37 (31)	30 (2.4)	
Sepsis and meningitis	80 (3.3)	43 (3.6)	37 (3)	
Hospitalization, n (%)	507 (21)	269 (22.7)	238 (19.4)	0.047
Intensive care need, n (%)	58 (2.4)	38 (3.2)	20 (1.6)	0.018
Mortality, n (%)	12 (0.5)	7 (0.6)	5 (0.4)	0.523

S. pneumonia: *Streptococcus pneumonia*; RTI: respiratory tract infection.

the *S. pneumoniae*-positive group than in the negative group ($p < 0.001$). When stratified by age, pneumococcal carriage was significantly more common in children under 12 months and significantly less common in those over 60 months of age ($p < 0.001$).

Pneumococcal positivity was significantly lower in fully vaccinated children than in incompletely vaccinated or unvaccinated children ($p < 0.001$). It was also lower among patients with underlying diseases than among those without ($p < 0.001$). However, among patients with comorbidities, those with hematological, oncological, and nephrological conditions had significantly higher rates of pneumococcal carriage ($p < 0.001$).

Infection categories and clinical outcomes

The infection-related parameters of all patients were examined in detail (Table 1). Analysis of infection categories revealed that upper RTIs were more frequent in the *S. pneumoniae*-negative group, whereas lower RTIs were significantly more common in the *S. pneumoniae*-positive group ($p = 0.009$). No cases of invasive pneumococcal disease were observed.

The overall hospitalization rate was 21% (n = 507), and ICU admission was required in 2.4% (n = 58) of the patients. Both outcomes were significantly more frequent in the *S. pneumoniae*-positive group than in the negative group (hospitalization: $p = 0.047$; ICU: $p = 0.018$). The overall mortality rate was 0.5%, with no significant intergroup differences.

Viral coinfections

The most frequently detected viruses among all patients were AdV (17.2%), influenza B (9%), SARS-CoV-2 (5.3%), RSV (4.9%), and influenza A (2.8%). The most common viruses in the pneumococcus-positive group were AdV (19.7%, n = 233), RSV (5.2%, n = 62), SARS-CoV-2 (5.1%, n = 60), influenza A (3.4%, n = 40), and influenza B (11%, n = 130) (Table 2). AdV, influenza B, and RSV were significantly more common in the *S. pneumoniae*-positive group ($p < 0.001$ for each). In contrast, enterovirus/rhinovirus positivity was significantly more common in the *S. pneumoniae*-negative group ($p = 0.039$). Viruses with significantly higher hospitalization rates were influenza B ($p < 0.001$), RSV ($p = 0.001$), bocavirus ($p = 0.001$), AdV ($p = 0.012$), and PIV 3 ($p = 0.04$).

Age and infection category distribution of viruses

Significant differences in the distribution of viral agents according to age group were detected for 4 viruses. AdV was detected at a higher rate in patients who were > 12 months of age ($p < 0.001$), RSV and SARS-CoV-2 were detected at a higher rate in patients < 24 months of age ($p < 0.001$), and influenza B was detected at a higher rate in patients > 60 months of age ($p < 0.001$). When the distribution of viral agents was analyzed according to the final infection category, a significant statistical difference was found for 5 viruses. Bocavirus ($p = 0.08$), RSV ($p < 0.001$), and human metapneumovirus ($p = 0.009$) were identified significantly more frequently in patients with lower RTI. AdV was significantly more frequent in patients with conjunctivitis ($p < 0.001$), and influenza B was significantly more frequent in patients with upper RTI,

Table 2. Viral coinfection status of the patients.

Characteristics	Total n = 2414	*S. pneumonia* positive n = 1186	*S. pneumonia* negative n = 1228	*p* value
Adenovirus, n (%)	372 (15.4)	233 (19.6)	139 (11.3)	< 0.001
Bocavirus, n (%)	36 (1.5)	15 (1.3)	21 (1.7)	0.367
Coronavirus, n (%)				
229E	17 (0.7)	6 (0.5)	11 (0.9)	0.252
HKU1	14 (0.6)	9 (0.8)	5 (0.4)	0.255
NL63	0	0	0	–
OC43	10 (0.4)	3 (0.3)	7 (0.6)	0.343
Enterovirus/rhinovirus, n (%)	42 (1.7)	14 (1.2)	28 (2.3)	0.039
Human metapneumovirus, n (%)	10 (0.4)	8 (0.7)	2 (0.2)	0.061
Influenza virus A, n (%)	68 (2.8)	40 (3.4)	28 (2.3)	0.105
Influenza virus B, n (%)	224 (9.3)	130 (11)	94 (7.7)	0.005
Parainfluenza viruses, n (%)				
PIV 1	19 (0.8)	12 (1)	7 (0.6)	0.219
PIV 2	2 (0.1)	2 (0.2)	0	0.241
PIV 3	10 (0.4)	6 (0.5)	4 (0.3)	0.541
PIV 4	3 (0.1)	3 (0.3)	0	0.118
Parechovirus, n (%)	0	0	0	–
Respiratory syncytial virus A/B, n (%)	104 (4.3)	62 (5.2)	42 (3.4)	0.029
SARS-CoV-2, n (%)	136 (5.6)	60 (5.1)	76 (6.2)	0.229

S. pneumonia: *Streptococcus pneumonia*; PIV: parainfluenza virus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

gastroenteritis, and conjunctivitis ($p < 0.001$).

Logistic regression analysis

A binary logistic regression model was used to identify variables that were independently associated with pneumococcal carriage (Figure 2). The following variables were independently associated with increased risk: frequency of AdV positivity, 2.1-fold (95% CI: 1.667, 2.670); frequency of intensive care need, 1.8-fold (95% CI: 1.021, 3.250); frequency of incompletely vaccinated or unvaccinated status, 1.7-fold (95% CI: 1.404, 2.149); frequency of influenza B positivity, 1.6-fold (95% CI: 1.267, 2.242); frequency of lower RTI, 1.3-fold (95% CI: 1.055, 1.618); and frequency of no underlying disease, 1.3-fold (95% CI: 1.099, 1.692). RSV positivity showed a 1.4-fold increased risk of pneumococcal carriage (OR: 1.4; 95% CI: 0.950–2.184), but the association was not statistically significant ($p = 0.086$).

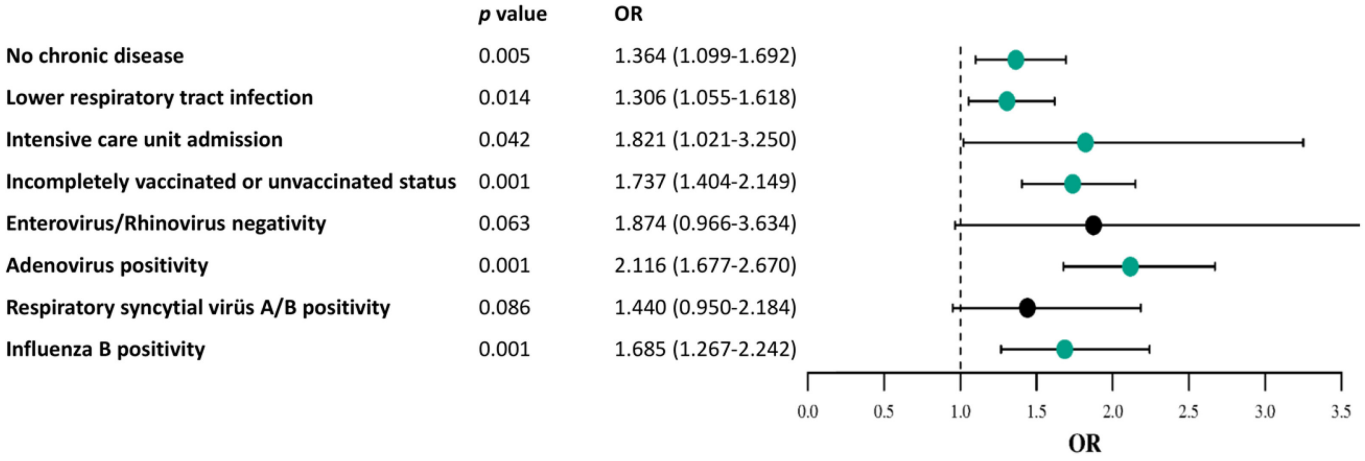
Discussion

The microbiome of the upper respiratory tract, particularly the nasopharynx, is a dynamic environment that undergoes notable shifts between periods of health and disease. Although viruses are often not the primary focus of microbiome studies, they exert significant temporal effects on the composition and stability of this microbial community through direct and indirect mechanisms [20]. Although *S. pneumoniae* is regarded as a transient or periodic resident of the nasopharynx, it exists in a complex and variable interplay with other microbial components, including viruses and fungi. This interaction is not static but fluctuates based on factors such as age, season, and other physiological and environmental conditions [20,21].

In this retrospective study, the epidemiological co-detection of pneumococcal carriage and respiratory viruses in children presenting with RTI symptoms was explored. Rather than implying a causal relationship, the findings describe the patterns of coexistence between NPC and specific viral agents during acute illness. Although a definitive causal link has yet to be established, previously published data suggest a possible synergistic interaction between pneumococcus and viral infections [11–16]. The interaction is likely bidirectional, as various studies have reported differing results: poses and intensity of NPC may facilitate the development of viral infection, while viruses may facilitate NPC and increase the risk of IPD [13–17,22,23]. However, the present study was not designed to evaluate this causality. The findings revealed both the diversity and frequency of simultaneously detected viral agents in children with NPC compared to those without, and explored the underlying factors that might influence these differences.

In this study, the overall NPC rate was 15.7%. It is well known that NPC rates vary depending on factors such as geographic location, country, age, underlying conditions, and antibiotic use [2]. Some studies have also shown that pneumococcal carriage density increases in the presence of upper or lower RTI symptoms [13,14,24]. As no previous studies in Türkiye have used a comparable methodology, interpreting the observed carriage rate remains challenging. The most expected result of this study was that the mean age of patients with *S. pneumoniae* positivity was significantly lower than that of patients without. This supports the well-established understanding that children under the age of 5 years, particularly those under 2 years, are the primary

Figure 2. Regression analysis of variables that resulted in statistically significantly higher values in the pneumococcal-positive group.



OR: Odds ratio (95% confidence interval). The age variable was not included in the model because age and vaccination status are very strongly correlated.

reservoirs of pneumococcal colonization [2,3]. In line with this, age-based subgroup analysis showed the lowest carriage rate in children over 60 months of age and the highest in those under 1 year of age.

This study also examined additional factors, beyond age, that may influence carriage, including pneumococcal vaccination status, presence of underlying diseases, and recent antibiotic use [25]. Antibiotic use did not significantly affect the carriage rate in this cohort. However, not being fully vaccinated against pneumococcus and the absence of underlying diseases were independently associated with pneumococcal carriage. The finding that the absence of underlying diseases was independently associated with pneumococcal carriage may appear counterintuitive. This association likely reflects healthcare-seeking and testing patterns rather than a true biological effect of the disease. Previously healthy children presenting with acute viral symptoms may be more likely to undergo multiplex respiratory testing, whereas children with chronic conditions may receive empirical treatment or targeted tests. Therefore, this result should be interpreted with caution and does not suggest that the underlying disease protects against pneumococcal carriage. While global studies following the introduction of PCVs have documented a shift toward non-vaccine serotypes and reductions in both IPD and non-invasive pneumococcal infections, few reports consistent with the findings of this study suggest that vaccination may also have a protective effect against colonization [6,25,26].

Most published NPC studies have focused on healthy children, and those investigating children with chronic diseases remain limited in number and diversity [27,28]. Available studies generally report higher carriage rates in children with chronic conditions. The findings in this study not only confirm this trend, but also identify specific underlying diseases that contribute to the increased NPC rate.

Analysis by the final infection category revealed a significantly higher frequency of lower RTIs among children with pneumococcal positivity, with an associated 1.3-fold increased risk of NPC. Colonization of the nasopharynx by pneumococcus is recognized as an early step in the pathogenesis of invasive pneumococcal disease [2,3]. Although the link between NPC and AOM is well established, the association with pneumonia is less clear [2,29]. Vaccination against pneumococcus has been associated with a reduction in all-cause pneumonia, and the global implementation of PCVs has led to substantial declines in pneumonia-related hospitalizations [30,31]. In this study, although lower RTIs were significantly more common in the pneumococcus-positive group, the expected difference in AOM rates was not observed between the groups. Similarly, hospitalization and intensive care unit admission were more frequently observed among pneumococcus-positive patients; however, the temporal and causal relationships between carriage and disease severity could not be determined. Importantly, pneumococcal detection in this study was based on qualitative RT-qPCR results without quantitative bacterial load or Ct-based density data. Therefore, PCR positivity reflects detection rather than colonization density or invasive potential, and these associations should be interpreted with appropriate caution. Although hospitalization and ICU admission were more frequent among pneumococcus-positive patients, these findings should be interpreted as associations rather than evidence of causality. The temporal sequence between viral infection, pneumococcal carriage, and clinical deterioration could not be determined in this retrospective design, and testing bias as well as underlying disease severity may have contributed to these associations.

The study period coincided with the winter and spring seasons in the Northern Hemisphere, and the most commonly detected viral agents among all patients were AdV, influenza viruses, SARS-CoV-2, and RSV. Among pneumococcus-positive children, the most frequently identified viruses were AdV, influenza viruses, RSV, and SARS-CoV-2, mirroring the overall distribution. When comparing groups based on pneumococcal carriage status, AdV, influenza B, and RSV were significantly more common in those with pneumococcal positivity. Previous studies have reported positive associations between NPC and various respiratory viruses, particularly rhinovirus, influenza virus, RSV, and parainfluenza virus (PIV) [6,14,15]. The associations with human metapneumovirus and AdV have been reported to be weaker, and the link with SARS-CoV-2 remains uncertain [6,15,23].

Although RSV and influenza viruses are recognized as facilitators of IPD, some studies have suggested an inverse relationship between RSV and NPC [7,12,32]. A study by Greenberg *et al*. examined the relationship between NPC during childhood community-acquired pneumonia and co-infecting viruses, and reported that the carriage rates were interestingly detected as lower in the RSV (+) pneumonia group, depending on the pneumococcal serotype [32]. In the same study, influenza viruses, AdV, and PIV were also examined, and no significant relationship was found between NPC

and these viruses. This study found no significant association between NPC and influenza, AdV, or PIV. Similarly, a study conducted in Bhutan during the summer months found a comparable distribution of respiratory viruses among children with and without NPC, despite using a diagnostic kit with a viral spectrum similar to that of the present study [33]. In that study, rhinovirus was more commonly detected in children without NPC, and RSV was more common in the NPC group, although these differences were not statistically significant. In another recent Brazilian study, rhinovirus was found more frequently among pneumococcal carriers, whereas the presence of human bocavirus alone was associated with the absence of NPC [34].

A recent case-control study by Wada *et al*. examined children under 5 years of age hospitalized with lower RTI and compared them to age-matched controls hospitalized for other reasons [35]. Pneumococcal colonization rates were 54.8% and 47.6% in the case and control groups, respectively, with no significant differences. However, AdV, RSV B, and rhinovirus were more prevalent in pneumococcus-positive cases, and a significant association was reported for these viruses in children aged < 5 years in the study population. Some studies have demonstrated a positive association between NPC and the detection of multiple viruses. Additionally, studies such as that by Brealey *et al*., conducted in children under 2 years of age with acute RTI, reported a positive association between NPC and multiple viral agents [36]. Notably, while RSV and influenza are frequently discussed in relation to NPC-virus interactions, AdV is rarely emphasized [37]. Interestingly, in the present study, AdV was the most frequently associated virus in NPC-virus co-detection. Furthermore, AdV detection showed a strong independent association with pneumococcal carriage in multivariable analysis, with a higher coefficient than that of influenza B. Although seasonal variation and local epidemiological factors may partly explain this finding, it is important to note that AdV is considered a nonseasonal virus, which may account for its consistent presence.

Although the present findings are insufficient to inform changes in clinical management or antimicrobial decision-making, they highlight the frequent coexistence of pneumococcal carriage and respiratory viral infections in pediatric patients, particularly those requiring hospitalization or intensive care. Awareness of bacterial–viral co-detection may aid clinicians in interpreting multiplex molecular diagnostic results and underscores the importance of vaccination against pneumococcus, especially in high-risk pediatric populations.

This study had several important limitations. Its retrospective, single-center design limits its generalizability and precludes causal inference. Pneumococcal serotyping and quantitative bacterial load measurements were not performed, which restricted the interpretation of vaccine effects and serotype-specific viral interactions. Additionally, not all potential confounding variables, including socioeconomic factors, viral load, and precise timing of symptom onset, could be assessed. The use of combined naso-oropharyngeal swabs instead of isolated nasopharyngeal sampling may have influenced carriage estimates and limited direct comparison with studies using strict nasopharyngeal specimens. Finally, the study focused on symptomatic children and did not include healthy controls, limiting comparisons with the baseline carriage rates. Overall, these findings describe patterns of coexistence rather than providing evidence of causal bacterial–viral interactions.

Conclusions

This large-scale, real-world molecular epidemiology study demonstrates that NPC frequently co-occurs with respiratory viral infections in children, with notable associations observed with adenovirus, influenza B, and RSV. These findings reflect epidemiological coexistence rather than causality and should be interpreted within the constraints of the study's design. Future prospective studies incorporating pneumococcal serotyping, bacterial density measurements, and longitudinal sampling are needed to clarify the biological mechanisms and clinical significance of bacterial–viral interactions in pediatric respiratory infections.

Ethics committee approval

Ethical approval for this study was obtained from the Medical Research Scientific and Ethical Review Board of Ankara Bilkent City Hospital (approval date: 29 May 2024; approval number: TABED 2-24-212).

Authors' contributions

All the authors contributed to the conception and design of the study. SKY, SYS, SASG, MYT, material preparation, data collection, and analysis; SKY, SA, manuscript—first draft. HKK, SÖ, TE, BG, AY, project conceptualization, analysis, and interpretation of the manuscript; ANÖP, BD, GİB, data interpretation, manuscript—critical review. All authors have commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

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Conflict of interest
No conflict of interest is declared.

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