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# Epidemiology and Demographic Patterns of Co-infections with Eight Respiratory Pathogens: A Large-Scale Retrospective Study

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**Abstract:** *Background*: Respiratory tract infections (RTIs) pose a significant global health burden, with etiology complicated by frequent pathogen co-infections. Comprehensive data on the epidemiology of co-infections across broad age and demographic spectra remain limited. *Methods*: This large-scale retrospective study analyzed 9,908 patients with suspected RTIs at a single hospital. Detection of IgM antibodies against eight common pathogens (Influenza A/B, Respiratory syncytial virus, Adenovirus, *Mycoplasma pneumoniae*, Parainfluenza virus, Coxsackievirus B, *Chlamydia pneumoniae*) was performed using rapid colloidal gold kits. Positivity rates, co-infection patterns, and their associations with age and gender were analyzed. *Results*: The overall pathogen detection rate was 33.1%. Influenza A was the most frequently detected pathogen overall, while Influenza B was most common among single infections. Notably, 33.1% of positive cases involved co-infections, with dual infections being most prevalent. The most frequent co-infection pattern was Influenza A with RSV. Age-stratified analysis revealed the highest proportion of co-infections in preschool children (48.3%) and older adults (40.3%), with adults having the lowest rate (28.5%). Significant gender-based differences were observed within specific age groups, such as higher Adenovirus rates in adult males and higher Mycoplasma pneumoniae rates in adult females. *Conclusion*: This study highlights the high frequency and specific demographic patterns of respiratory pathogen co-infections, underscoring the complexity of RTI etiology. The findings emphasize the need for considering multiple pathogens in clinical diagnosis and suggest that both age and gender may influence infection susceptibility and co-infection risk, informing future surveillance and management strategies.

**Keywords:** Respiratory virus; Co-infection; IgM antibody; Multiplex panels detection

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# 1. Introduction

Respiratory tract infections (RTIs) are a leading cause of global morbidity and mortality, imposing a substantial burden on healthcare systems worldwide. Notably, lower respiratory infections rank among the top five causes contributing to global mortality inequality [1]. The clinical presentation of RTIs is often nonspecific, yet they can be caused by a diverse spectrum of pathogens, including influenza viruses (Flu A, Flu B), respiratory syncytial virus (RSV), adenovirus (ADV), *Mycoplasma pneumoniae* (MP), parainfluenza virus (PIV), coxsackievirus B (COXB), *Chlamydia pneumoniae* (CP) and others [2,3]. Accurate and timely etiological diagnosis is crucial for guiding appropriate clinical management, infection control measures, and understanding epidemiological trends [4].

A significant challenge in managing and studying RTIs is pathogen co-infection, where multiple pathogens are detected simultaneously. Co-infections are common and may alter disease severity, complicate presentation, and impact outcomes [5]. For instance, RSV co-infection with other viruses increases the risk of asthma exacerbation in young children, and viral co-infections in children with SARS-CoV-2 have been associated with severe outcomes [5,6]. While some studies describe age and seasonal variations in single pathogen epidemiology, comprehensive data on co-infection patterns across broad demographic strata, including age and gender, remain limited [3,7,8]. Existing research often focuses on specific subgroups, such as hospitalized children, leaving gaps in understanding co-infection dynamics across the entire age spectrum and in outpatient settings [7,8]. Furthermore, while rapid point-of-care diagnostics like colloidal gold-based IgM assays are widely used in clinical practice, studies reflecting real-world pathogen detection profiles from such methods are needed.

To address these critical knowledge gaps, we conducted a large-scale retrospective study. The primary aim was to elucidate the detection profile of eight common respiratory pathogens via rapid IgM assays in a cohort of patients with suspected RTIs, determining both overall and pathogen-specific positivity rates. Furthermore, we sought to characterize the frequency, distribution, and specific combinatorial patterns of pathogen co-infections. Finally, we aimed to assess whether the rates of single infections, co-infections, and infection by specific pathogens varied significantly across different age groups and between genders. By investigating these aspects, our findings are intended to offer valuable insights for refining diagnostic approaches and enhancing the understanding of the complex epidemiology of respiratory pathogens in a diverse clinical population.

# 2. Methods

## 2.1. Study design and population

This retrospective study included 9,908 patients with suspected respiratory tract infections who underwent testing for eight respiratory pathogens (Flu A, Flu B, ADV, MP, PIV, RSV, COXB, CP) at West China Fourth Hospital of Sichuan University from January 1, 2021, to August 31, 2025. The cohort comprised 6838 hospitalized patients and 3070 outpatients. There were 5609 males and 4298 females, with an age range of 2–102 years and a median age of 54 years.

## 2.2. Pathogen detection

Testing was performed using two commercially available colloidal gold-based rapid IgM antibody detection kits (Innovita, China). The "Respiratory Virus Three" kit was used for the qualitative detection of IgM antibodies against Influenza A virus, Influenza B virus, and Parainfluenza virus. The "Respiratory Virus Five" kit was used for the qualitative detection of IgM antibodies against Mycoplasma pneumoniae, Chlamydia pneumoniae, Respiratory Syncytial Virus, Adenovirus, and Coxsackievirus B group. Both kits operate on an immunocapture principle, where the presence of pathogen-specific IgM antibodies in serum or whole blood samples leads to the formation of a visible purple band at the corresponding test line (T) on the nitrocellulose membrane. All testing and interpretation of results were performed strictly according to the manufacturers' instructions, with results read within 15–25 minutes of sample application.

## 2.3. Statistical analysis

All statistical analyses were performed using R software (version 4.4.2). Descriptive statistics were used to summarize patient demographics and the detection rates (positive rates) for each individual pathogen. Co-infection was defined as the simultaneous detection of IgM antibodies for two or more pathogens in a single patient. The frequency and patterns of co-infections were analyzed. Group comparisons (e.g., positive rates between genders or across different age groups) were conducted using the Chi-square test or Fisher's exact test, as appropriate. A two-sided p-value of less than 0.05 was considered statistically significant.

## 2.4. Ethical considerations

This retrospective study was approved by the Ethics Committee at West China Fourth Hospital of Sichuan University (Approval No. HXSY-EC-2023025). The requirement for written informed consent was waived, as the study involved the analysis of anonymized, pre-existing clinical data and did not affect patient welfare or rights. All procedures were conducted in accordance with the relevant guidelines and regulations.

# 3. Results

Among the eight respiratory pathogens tested (Flu A, Flu B, ADV, MP, PIV, RSV, COXB, CP), a total of 3,277 individuals tested positive, yielding an overall positivity rate of 33.1%. Of these positive cases, 2,191 were attributed to single pathogen positivity, while 1,086 cases demonstrated co-infection. The distribution of co-infections was as follows: 790 cases of dual positivity, 224 cases of triple positivity, 61 cases of quadruple positivity, 8 cases of quintuple positivity, and 3 cases of sextuple positivity. The overall detection rates for each specific pathogen, along with their respective frequencies in single positivity and co-infection, are detailed in **Table 1**. The data reveal that Flu A exhibited the highest overall detection rate among the eight pathogens. However, within the subset of single positivity cases, Flu B constituted the largest proportion. Notably, similar to Flu A, RSV demonstrated a significantly lower rate of single infection compared to its frequency of co-occurrence with other pathogens.

**Table 1.** Prevalence of IgM antibodies against respiratory pathogens

| Pathogen | Total positive n (%) | Detected alone n (%) | Detected in co-infection n (%) |
|---|---|---|---|
| Flu A | 1475 (14.89) | 628 (6.34) | 847 (8.55) |
| Flu B | 1153 (11.64) | 706 (7.13) | 447 (4.51) |
| MP | 940 (9.49) | 564 (5.69) | 376 (3.79) |
| RSV | 685 (6.91) | 132 (1.33) | 553 (5.58) |
| ADV | 277 (2.80) | 73 (0.74) | 204 (2.06) |
| COXB | 109 (1.10) | 37 (0.37) | 72 (0.73) |
| PIV | 93 (0.94) | 45 (0.45) | 48 (0.48) |
| CP | 13 (0.13) | 6 (0.06) | 7 (0.07) |

Subsequent analysis stratified by age and gender further revealed the incidence rates of each pathogen among populations with different demographic characteristics (**Table 2**). The age stratification criteria were defined as follows: infants and preschool children (2–5 years old), school-age children and adolescents (6–17 years old), adults (18–64 years old), older adults (65–79 years old), and the oldest-old (80–102 years old). The results indicate that the preschool children group accounted for a relatively small proportion of the entire study population, and their results should be interpreted with caution. In the school-age children and adolescents' group, the infection rates of Flu B and MP were relatively higher compared to other age groups, while infections with PIV, RSV, COXB, CP, and ADV were less common. Similarly, in the oldest-old group, infections with PIV, COXB, and CP was also relatively uncommon.

**Table 2.** Pathogen-specific infection rates stratified by age and gender

| Age | Gender | Total | Flu A n (%) | Flu B n (%) | ADV n (%) | MP n (%) | PIV n (%) | RSV n (%) | COXB n (%) | CP n (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 2–5 | Female | 20 | 4 (20) | 7 (35) | 0 (0) | 9 (45) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | Male | 26 | 4 (15.38) | 9 (34.62) | 0 (0) | 13 (50) | 1 (3.85) | 0 (0) | 0 (0) | 0 (0) |
| 6–17 | Female | 121 | 14 (11.57) | 31 (25.62) | 0 (0) | 41 (33.88) | 0 (0) | 0 (0) | 0 (0) | 1 (0.83) |
| | Male | 143 | 16 (11.19) | 29 (20.28) | 1 (0.7) | 54 (37.76) | 0 (0) | 1 (0.7) | 0 (0) | 1 (0.7) |
| 18–64 | Female | 2697 | 357 (13.24) | 257 (9.53) | 50 (1.85) | 396 (14.68) | 19 (0.7) | 96 (3.56) | 33 (1.22) | 3 (0.11) |
| | Male | 3291 | 420 (12.76) | 287 (8.72) | 99 (3.01) | 272 (8.26) | 30 (0.91) | 177 (5.38) | 32 (0.97) | 5 (0.15) |
| 65–79 | Female | 862 | 129 (14.97) | 148 (17.17) | 24 (2.78) | 47 (5.45) | 12 (1.39) | 84 (9.74) | 14 (1.62) | 1 (0.12) |
| | Male | 1476 | 312 (21.14) | 173 (11.72) | 54 (3.66) | 61 (4.13) | 11 (0.75) | 192 (13.01) | 19 (1.29) | 1 (0.07) |
| 80–102 | Female | 597 | 105 (17.59) | 91 (15.24) | 19 (3.18) | 17 (2.85) | 7 (1.17) | 65 (10.89) | 4 (0.67) | 0 (0) |
| | Male | 672 | 114 (16.96) | 121 (18.01) | 30 (4.46) | 29 (4.32) | 13 (1.93) | 70 (10.42) | 7 (1.04) | 1 (0.15) |

Chi-square tests conducted across age and gender strata revealed statistically significant differences in pathogen positivity rates between males and females within specific age groups. Among adults, significant gender-based disparities were observed for ADV and MP, with males showing a higher infection rate of ADV, while females exhibited a significantly higher positivity rate for MP. In the elderly population, significant differences were also found for Flu A and RSV, with males demonstrating higher infection rates for both pathogens compared to females.

Subsequent analysis of co-infection patterns and their proportional distribution among all co-infected cases revealed that dual infection with Flu A and RSV was the most frequent combination. This was followed by Flu B and MP, as well as Flu A and Flu B. Among triple infection patterns, the combination of ADV, Flu A, and RSV was the most common, followed by Flu A, Flu B, and RSV, and then Flu A, MP, and RSV. For quadruple infections, the pattern ADV, Flu A, Flu B, and RSV ranked first in frequency, succeeded by ADV, Flu A, MP, and RSV and Flu A, Flu B, MP, and RSV. Further details are provided in **Table 3**.

**Table 3.** Patterns and frequency of multiple pathogen co-infection

| Pattern | Count | Percentage (%) | Pattern | Count | Percentage (%) |
|---|---|---|---|---|---|
| Flu A, RSV | 270 | 8.24 | CP, PIV | 1 | 0.03 |
| Flu B, MP | 138 | 4.21 | ADV, Flu A, RSV | 78 | 2.38 |
| Flu A, Flu B | 130 | 3.97 | Flu A, Flu B, RSV | 53 | 1.62 |
| Flu A, MP | 108 | 3.30 | Flu A, MP, RSV | 30 | 0.92 |
| ADV, Flu A | 33 | 1.01 | Flu A, Flu B, MP | 24 | 0.73 |
| ADV, Flu B | 14 | 0.43 | COXB, Flu A, RSV | 9 | 0.27 |
| Flu B, RSV | 14 | 0.43 | COXB, Flu A, Flu B | 4 | 0.12 |
| COXB, Flu A | 12 | 0.37 | Flu A, PIV, RSV | 4 | 0.12 |
| COXB, MP | 11 | 0.34 | ADV, Flu A, Flu B | 3 | 0.09 |
| Flu A, PIV | 10 | 0.31 | COXB, Flu B, MP | 3 | 0.09 |
| ADV, RSV | 9 | 0.27 | ADV, Flu A, MP | 2 | 0.06 |
| COXB, Flu B | 8 | 0.24 | ADV, Flu B, MP | 2 | 0.06 |
| MP, PIV | 6 | 0.18 | ADV, MP, RSV | 2 | 0.06 |
| MP, RSV | 6 | 0.18 | COXB, Flu A, MP | 2 | 0.06 |
| Flu B, PIV | 5 | 0.15 | Flu A, Flu B, PIV | 2 | 0.06 |
| ADV, MP | 3 | 0.09 | ADV, COXB, RSV | 1 | 0.03 |
| COXB, RSV | 3 | 0.09 | COXB, Flu B, RSV | 1 | 0.03 |
| PIV, RSV | 3 | 0.09 | CP, Flu B, PIV | 1 | 0.03 |
| ADV, PIV | 2 | 0.06 | Flu A, MP, PIV | 1 | 0.03 |
| CP, Flu A | 2 | 0.06 | Flu B, MP, PIV | 1 | 0.03 |
| COXB, PIV | 1 | 0.03 | Flu B, MP, RSV | 1 | 0.03 |
| CP, Flu B | 1 | 0.03 | | | |

**Table 3 (continued).** Patterns and frequency of multiple pathogen co-infection

| Pattern | Count | Percentage (%) |
|---|---|---|
| ADV, Flu A, Flu B, RSV | 19 | 0.58 |
| ADV, Flu A, MP, RSV | 14 | 0.43 |
| Flu A, Flu B, MP, RSV | 9 | 0.27 |
| ADV, COXB, Flu A, RSV | 6 | 0.18 |
| ADV, COXB, Flu A, Flu B | 2 | 0.06 |
| ADV, Flu A, PIV, RSV | 2 | 0.06 |
| COXB, Flu A, Flu B, RSV | 2 | 0.06 |
| Flu A, MP, PIV, RSV | 2 | 0.06 |
| ADV, COXB, MP, RSV | 1 | 0.03 |
| ADV, Flu B, MP, RSV | 1 | 0.03 |
| COXB, Flu A, MP, RSV | 1 | 0.03 |

**Table 3 (Continued)**

| Pattern | Count | Percentage (%) |
|---|---|---|
| CP, Flu A, PIV, RSV | 1 | 0.03 |
| Flu A, Flu B, PIV, RSV | 1 | 0.03 |
| ADV, Flu A, Flu B, MP, RSV | 3 | 0.09 |
| ADV, COXB, CP, Flu A, PIV | 1 | 0.03 |
| ADV, COXB, Flu A, Flu B, RSV | 1 | 0.03 |
| ADV, COXB, Flu A, PIV, RSV | 1 | 0.03 |
| ADV, Flu A, MP, PIV, RSV | 1 | 0.03 |
| Flu A, Flu B, MP, PIV, RSV | 1 | 0.03 |
| ADV, COXB, Flu A, Flu B, MP, RSV | 2 | 0.06 |
| ADV, Flu A, Flu B, MP, PIV, RSV | 1 | 0.03 |

The statistical results regarding single and co-infection patterns across different age groups reveal that the proportion of co-infections among total infection cases was highest in preschool children (48.28%), followed by older adults (40.25%). In contrast, adults exhibited the lowest proportion of co-infections (28.52%). See more details in **Table 4**.

**Table 4.** Composition of single and co-infections by age group

| Age | Total positive | Detected alone n (%) | Detected in co-infection n (%) |
|---|---|---|---|
| 2–5 | 29 | 15 (51.72) | 14 (48.28) |
| 6–17 | 135 | 83 (61.48) | 52 (38.52) |
| 18–64 | 1834 | 1311 (71.48) | 523 (28.52) |
| 65–79 | 815 | 487 (59.75) | 328 (40.25) |
| 80–102 | 463 | 294 (63.50) | 169 (36.50) |

The overall chi-square test comparing the composition of infection types across age groups yielded a significant result ($\chi^2 = 43.87$, $p = 2.45 \times 10^{-8}$). This indicates a statistically significant difference in the distribution of single versus co-infections among the different age strata. Post-hoc pairwise comparisons with Bonferroni correction revealed specific significant differences between the following groups: adults versus the oldest-old ($p = 0.015$), and adults versus the older adults ($p = 4.79 \times 10^{-8}$.

## 4. Discussion

This large-scale retrospective study delineates a detailed epidemiological profile of eight common respiratory pathogens detected via rapid IgM assays in a diverse cohort of 9,908 patients with suspected RTIs. The overall pathogen positivity rate of 33.1% aligns with the broad spectrum reported in multi-pathogen surveillance studies, which varies considerably based on population, setting, and diagnostic methodology [9,10]. Flu A was identified as the most frequently detected pathogen overall, consistent with its established role as a major cause of seasonal respiratory morbidity. A notable finding was the predominance of Flu B among cases of single pathogen detection, which may reflect specific local circulation dynamics or period-specific susceptibility patterns warranting further investigation.

A central and significant finding is the high frequency of pathogen co-detection, with one-third of all positive cases involving multiple pathogens. This underscores the etiological complexity of RTIs and highlights a critical limitation of single-pathogen diagnostic approaches in clinical practice. Dual infections were the most common co-infection type. The most frequent specific combination was Flu A with RSV, a pattern previously observed that raises important questions about potential viral interference, altered clinical severity, or overlapping seasonal peaks [11,12]. Other prevalent patterns, such as Flu B with MP and Flu A with Flu B, illustrate the common mixing of viral and atypical bacterial pathogens, as well as co-circulation within virus families. These patterns suggest that in clinical scenarios where influenza is suspected, concurrent testing for RSV and MP should be strongly considered, and vice versa [13].

Stratified analysis revealed crucial demographic patterns. The proportion of co-infections among all positive cases was highest in preschool children (2–5 years) at 48.3%, followed by older adults (65–79 years) at 40.3%. This is consistent with the known higher frequency of multiple pathogen exposures in young children due to immature immunity and dense social contact, and in older adults potentially due to immunosenescence and comorbid conditions [13,14]. In contrast, adults exhibited the lowest co-infection proportion (28.5%), a statistically significant difference confirmed by post-hoc analysis, likely reflecting more robust immune function and different exposure networks.

Our analysis also revealed statistically significant gender-based differences in pathogen positivity within specific age groups. Among adults, males had a higher infection rate of ADV, while females exhibited a significantly higher positivity rate for MP. In the elderly population, males demonstrated higher infection rates for both Flu A and RSV. These findings contrast with some previous reports which concluded that respiratory pathogen infection rates vary primarily by age and season, but not by gender [15,16]. However, they align with other studies noting gender disparities, such as higher MP and ADV rates in boys within pediatric cohorts [17]. It is important to note that the pediatric subgroup (2–17 years) in our study constituted a relatively small proportion of the total positive cases. Therefore, conclusions regarding infection patterns in children, while suggestive, should be interpreted with caution, and reference to larger, dedicated pediatric studies is recommended for more definitive insights [9,17,18].

Several limitations must be acknowledged to contextualize our findings. First, the reliance on IgM antibody detection, while offering rapid, point-of-care utility, has inherent constraints including potential cross-reactivity, interference from rheumatoid factor, and the inability to precisely distinguish between recent, past, or persistent infection within the serological window. This may lead to an overestimation of active simultaneous co-infections compared to direct molecular methods like PCR [10]. Second, as a single-center retrospective study, the generalizability of our findings to other geographical regions or healthcare settings may be limited. Third, the lack of detailed, prospectively collected clinical metadata (e.g., symptom severity, comorbidities, treatment, and outcomes) precluded analysis of the correlation between specific pathogen or co-infection patterns and disease severity, progression, or healthcare burden. Finally, our predefined panel, though covering major pathogens, did not include other common agents such as rhinovirus, SARS-CoV-2, metapneumovirus, or typical bacteria, which undoubtedly contributes to the overall etiology of RTIs and may lead to an underestimation of co-infection complexity [10,15] .

## 5. Conclusion

Despite these limitations, this study provides valuable real-world epidemiological data on the co-circulation and demographic distribution of key respiratory pathogens using a widely available diagnostic method. The findings emphasize the necessity of moving beyond single-pathogen diagnostic paradigms in both clinical and public health contexts. Future research should employ prospective, multi-center designs that integrate highly sensitive molecular multiplex panels (e.g., PCR-based syndromic testing) with comprehensive clinical and epidemiological data collection. This approach will enable a more precise determination of active co-infection rates and a robust assessment of their clinical and public health impact. Furthermore, investigating the immunological mechanisms, such as viral interference or synergistic pathogenesis, underlying high-frequency co-infection patterns like Flu A/RSV, is a crucial direction [12,13]. Ultimately, such refined insights are essential for developing more nuanced diagnostic algorithms, guiding targeted therapeutic and preventive strategies, and strengthening surveillance systems to mitigate the burden of respiratory infections across diverse populations.

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## Disclosure statement

The authors declare no conflict of interest.

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