

## Original Article

**RESPIRATORY PATHOGENS DETECTED BY MULTIPLEX PCR IN MALAYSIAN PUBLIC HOSPITALS: PREVALENCE AND EPIDEMIOLOGIC PATTERNS FROM JANUARY 2023 UNTIL JULY 2024**

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**ABSTRACT**

Respiratory tract infections remain an important cause of hospitalisation and diagnostic uncertainty, and multiplex polymerase chain reaction testing offers rapid detection of multiple pathogens in routine clinical practice. We aimed to describe the prevalence, demographic associations, and temporal trends of respiratory pathogens detected by the QIAstat-Dx Respiratory SARS-CoV-2 Panel in Malaysian public hospitals. This multicentre retrospective cross-sectional study included nasopharyngeal swab samples from cases with suspected acute respiratory infections tested between 1 January 2023 and 31 July 2024. Data were collected from participating sites using standardised record forms and analysed. We assessed overall positivity, pathogen frequencies, age-group and regional distributions, and monthly and quarterly trends. A total of 8,361 samples were included, of which 5,252 (62.8%) had at least one pathogen detected. Single detections were identified in 4,279 samples (51.2%), while co-detections were observed in 973 samples (11.6%). Viral pathogens predominated. Rhinovirus/enterovirus was the most frequently detected pathogen (23.8%), followed by respiratory syncytial virus A/B (13.7%) and influenza A H1N1/2009 (4.8%). Positivity was highest among children aged 1 to 4 years and infants aged 1 to 11 months, and age-related variation was more pronounced than regional variation. The leading pathogens also demonstrated distinct temporal profiles, with different peak months across the study period. In conclusion, respiratory pathogen detection in this hospital-based population was characterised by a high positivity rate, predominance of viral pathogens, marked age-related differences, and distinct temporal patterns. These findings support the value of multiplex PCR for respiratory pathogen surveillance in Malaysian public hospitals.

**KEYWORDS:** respiratory tract infections, multiplex PCR, rhinovirus/enterovirus, respiratory syncytial virus, epidemiology

## INTRODUCTION

Respiratory tract infections continue to impose a major global health burden and remain an important cause of illness, hospitalisation, and death across all age groups, particularly among young children. The Global Burden of Disease 2021 analysis estimated that lower respiratory infections not attributable to coronavirus disease 2019 (COVID-19) accounted for approximately 344 million incident episodes and 2.18 million deaths worldwide in 2021, including about 502,000 deaths among children younger than five years [1]. These findings highlight that such infections remain a substantial cause of preventable mortality despite long-term declines in death rates [1]. This burden is especially relevant in children, in whom respiratory infections contribute markedly to healthcare utilisation and hospital admission. A recent systematic review and meta-analysis of paediatric respiratory viral infections similarly showed that viral respiratory infections remain a major burden in children, with rhinovirus/enterovirus and respiratory syncytial virus (RSV) among the most frequently detected pathogens, including in hospitalised paediatric populations [2].

Accurate and timely identification of respiratory pathogens is important for clinical management, infection prevention, and epidemiologic surveillance. Multiplex polymerase chain reaction (PCR) and syndromic respiratory testing have become increasingly valuable in clinical microbiology because they enable rapid and broad detection of respiratory pathogens compared with conventional methods. A systematic review demonstrated high diagnostic accuracy for major respiratory viruses, while a later meta-analysis found that rapid multiplex PCR shortened time to results, reduced hospital stay, and improved antiviral use and infection control in hospital settings [3,4]. These findings support multiplex PCR not only as an accurate diagnostic tool but also as a clinically useful platform for improving patient management and supporting hospital-based surveillance.

This role has become even more relevant in the post-COVID-19 era, when respiratory pathogen epidemiology has become more dynamic. However, long-term data describing contemporary respiratory pathogen patterns remain limited, particularly in Southeast Asia and in multicentre hospital-based settings. In this context, multiplex PCR data can provide important insights into current pathogen distribution, co-detection patterns, demographic associations, and temporal trends. Therefore, this study aimed to describe the prevalence, distribution, co-detection patterns, demographic associations, and temporal trends of respiratory pathogens detected by multiplex PCR-based respiratory pathogen testing in Malaysian public hospitals.

## MATERIALS AND METHODS

### Study design and setting

This multicentre retrospective cross-sectional study included 34 Malaysian public hospitals nationwide. It included tertiary referral centres and district hospitals. These hospitals were categorised into six regions: Central, East, North, and South Peninsular Malaysia, Sabah, and Sarawak.

### Selection and description of participants

The study population consisted of individuals with suspected acute respiratory infections—including hospitalised patients, patients presenting to the Emergency Department, and forensic cases—from whom nasopharyngeal swab samples were collected and tested using the QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN, Hilden, Germany) between 1 January 2023 and 31 July 2024.

Samples were excluded if they: (1) had incomplete demographic information (missing or invalid sex or age); (2) returned an uninterpretable error result on the QIAstat analyser; or (3) were duplicate samples from the same patient collected within one week of the first sample. Invalid sex was defined as any entry other than 'Male' or 'Female'. Invalid age was defined as negative values, zero, or nil. Universal sampling was used, and all eligible samples collected during the study period were included.

### Technical information

Upper respiratory samples (collected using nasopharyngeal swabs in universal transport medium) from patients with suspected acute respiratory infections were submitted by treating clinicians for diagnostic testing as part of routine clinical care. Nasopharyngeal swabs collected during autopsy from forensic cases with suspected acute respiratory infection were similarly submitted as part of investigations into suspected respiratory infection during autopsy. All samples were tested using the QIAstat-Dx Respiratory SARS-CoV-2 Panel, a multiplex PCR assay, according to the manufacturer's instructions as part of routine hospital diagnostic workflows. During the study period, two panel configurations were in use across participating hospitals. The earlier configuration detected 22 respiratory pathogens, while the later 23-pathogen configuration, approved by the Malaysian Medical Device Authority and launched in Malaysia in June 2024, additionally included *Chlamydophila pneumoniae* [5]. The 23-pathogen configuration detects influenza A, influenza A subtype H1N1/2009, influenza A subtype H1, influenza A subtype H3, influenza B, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, respiratory syncytial virus A/B, human metapneumovirus A/B, adenovirus, bocavirus, rhinovirus/enterovirus, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis*. The 22-pathogen configuration comprised the same targets except for *C. pneumoniae*. Participating hospitals adopted the 23-pathogen configuration at different times; consequently, *C. pneumoniae* was not uniformly tested across the study population. To avoid bias from differential availability of this target, we excluded *C. pneumoniae* detections from pathogen-frequency and comparative analyses.

Each participating site accessed its laboratory records and completed a standardised data collection sheet. The following information was collected: type of test performed, sample date, detected pathogens, patient age, and patient gender. Only laboratory data were collected in this study. No archived specimens or routine clinical investigation biospecimens were used or processed. For the respiratory panel analysis, the main study variables were sample date, patient age, patient gender, and detected respiratory pathogens. The outcomes of interest included overall positivity, frequency of individual respiratory pathogens, co-detection frequency, and the distribution of pathogen detection by age group, region, ward, and time period. For pathogen-level analysis, samples positive for the generic influenza A target but negative for all subtype-specific targets (H1, H3, and H1N1/2009) were classified as influenza A (unsubtyped). When one or more subtype-specific targets (H1, H3, or H1N1/2009) were detected, the sample was classified by the detected subtype(s), regardless of whether the generic influenza A target was also detected.

### Statistics

Data cleaning, statistical analysis, and table and figure preparation were performed using Python 3.13.5 in a computational notebook environment. The main libraries used were pandas 2.2.3, NumPy 2.3.5, SciPy 1.17.0, statsmodels 0.14.6, and openpyxl 3.1.5. ChatGPT (OpenAI; GPT-5.4 Thinking) was used to assist with code drafting and language refinement of the statistical description; all analyses, results, and interpretations were reviewed and confirmed by the authors. Descriptive statistics were presented as frequencies and percentages. Positivity rate was defined as the proportion of samples

with at least one pathogen detected. Pearson's chi-square tests of independence were used to compare overall positivity across regions, age groups, sex, wards, and time periods, and to compare the distribution of major pathogens across age groups. Monthly and quarterly trends were summarised descriptively; calendar quarters were defined as Q1 = January–March, Q2 = April–June, Q3 = July–September, and Q4 = October–December. A  $P$ -value < 0.05 was considered statistically significant. Cramér's  $V$  was used to show the magnitude of association.

### Ethical approval

This research was approved by the Medical Research & Ethics Committee (MREC) under the National Medical Research Registry (NMRR ID-24-02180-DTT (IIR); approval letter reference: 24-02180-DTT (1); date: 2 September 2024). Patient consent was waived because this study involved retrospective review of de-identified laboratory records and did not affect patient management.

## RESULTS

A total of 8,361 respiratory samples were included in the final analysis, as shown in Figure 1. No samples were excluded due to analyser error results or duplicates within one week. Overall, 5,252 samples (62.8%) had at least one pathogen detected, while 3,109 (37.2%) had no target detected. Single detections were identified in 4,279 samples (51.2%), whereas co-detections were observed in 973 samples (11.6%). Viral targets predominated: 4,948 samples (59.2%) had at least one viral target detected, compared with 587 (7.0%) with at least one atypical bacterial target. Most samples were classified as viral-only ( $n = 4665$ ; 55.8%), while 304 (3.6%) were atypical bacterial-only and 283 (3.4%) had both viral and atypical bacterial targets.

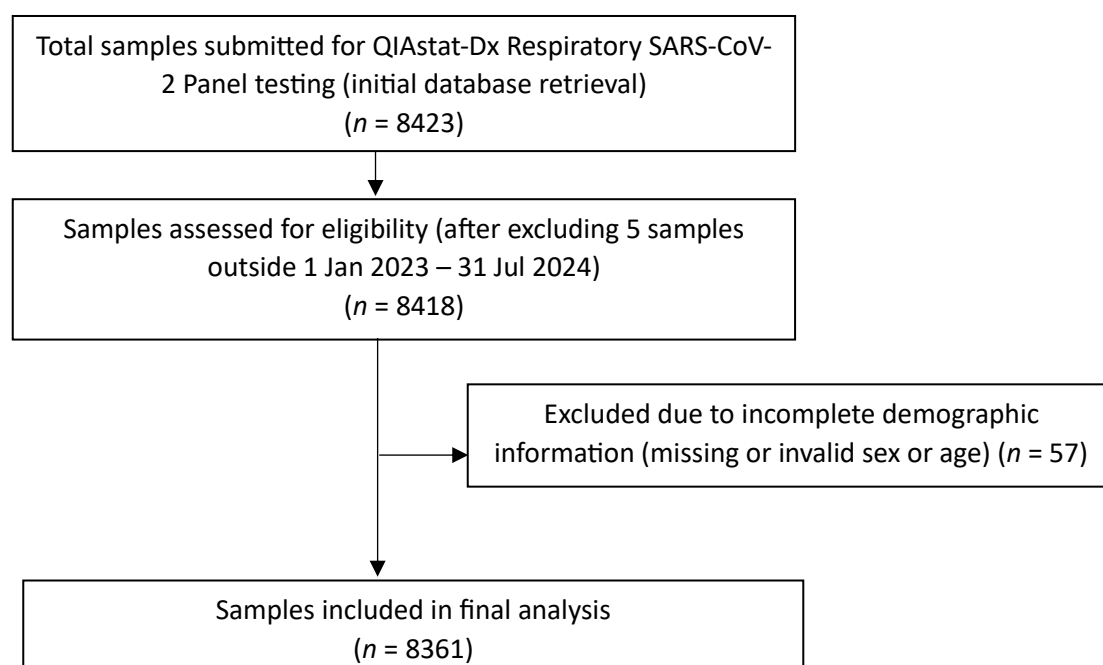


**Figure 1.** STROBE flowchart of the study. STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

**Table 1.** Characteristics of study samples and positivity by subgroup

| Characteristic              | Total samples, <i>N</i> (%) | Positive samples, <i>n</i> | Positivity, % | <i>P</i> -value | Effect size (Cramér's <i>V</i> ) |
|-----------------------------|-----------------------------|----------------------------|---------------|-----------------|----------------------------------|
| Overall                     | 8361 (100.0)                | 5252                       | 62.8          |                 |                                  |
| Region                      |                             |                            |               | <0.001          | 0.062                            |
| Central Peninsular Malaysia | 1155 (13.8)                 | 701                        | 60.7          |                 |                                  |
| East Peninsular Malaysia    | 998 (11.9)                  | 592                        | 59.3          |                 |                                  |
| North Peninsular Malaysia   | 1402 (16.8)                 | 825                        | 58.8          |                 |                                  |
| South Peninsular Malaysia   | 1973 (23.6)                 | 1297                       | 65.7          |                 |                                  |
| Sabah                       | 1187 (14.2)                 | 791                        | 66.6          |                 |                                  |
| Sarawak                     | 1646 (19.7)                 | 1046                       | 63.5          |                 |                                  |
| Age group                   |                             |                            |               | <0.001          | 0.285                            |
| <1 Month                    | 462 (5.5)                   | 218                        | 47.2          |                 |                                  |
| 1–11 Months                 | 2472 (29.6)                 | 1856                       | 75.1          |                 |                                  |
| 1–4 Years                   | 1712 (20.5)                 | 1327                       | 77.5          |                 |                                  |
| 5–17 Years                  | 1024 (12.2)                 | 595                        | 58.1          |                 |                                  |
| 18–64 Years                 | 2096 (25.1)                 | 994                        | 47.4          |                 |                                  |
| ≥65 Years                   | 595 (7.1)                   | 262                        | 44.0          |                 |                                  |
| Sex                         |                             |                            |               | <0.001          | 0.037                            |
| Female                      | 3920 (46.9)                 | 2537                       | 64.7          |                 |                                  |
| Male                        | 4441 (53.1)                 | 2715                       | 61.1          |                 |                                  |
| Year                        |                             |                            |               | <0.001          | 0.060                            |
| 2023                        | 4643 (55.5)                 | 3038                       | 65.4          |                 |                                  |
| 2024                        | 3718 (44.5)                 | 2214                       | 59.5          |                 |                                  |
| Ward                        |                             |                            |               | <0.001          | 0.242                            |
| Paediatric General Ward     | 3205 (38.3)                 | 2390                       | 74.6          |                 |                                  |
| Paediatric ICU              | 2267 (27.1)                 | 1478                       | 65.2          |                 |                                  |
| Adult General Ward          | 1281 (15.3)                 | 648                        | 50.6          |                 |                                  |
| Adult ICU                   | 1270 (15.2)                 | 558                        | 43.9          |                 |                                  |
| Emergency Department        | 312 (3.7)                   | 169                        | 54.2          |                 |                                  |
| Forensic                    | 26 (0.3)                    | 9                          | 34.6          |                 |                                  |
| Year-month                  |                             |                            |               | <0.001          | 0.088                            |
| 2023-01                     | 244 (2.9)                   | 175                        | 71.7          |                 |                                  |
| 2023-02                     | 213 (2.5)                   | 153                        | 71.8          |                 |                                  |
| 2023-03                     | 291 (3.5)                   | 193                        | 66.3          |                 |                                  |
| 2023-04                     | 315 (3.8)                   | 202                        | 64.1          |                 |                                  |
| 2023-05                     | 351 (4.2)                   | 238                        | 67.8          |                 |                                  |
| 2023-06                     | 285 (3.4)                   | 184                        | 64.6          |                 |                                  |
| 2023-07                     | 355 (4.2)                   | 237                        | 66.8          |                 |                                  |
| 2023-08                     | 495 (5.9)                   | 292                        | 59.0          |                 |                                  |
| 2023-09                     | 566 (6.8)                   | 379                        | 67.0          |                 |                                  |
| 2023-10                     | 508 (6.1)                   | 314                        | 61.8          |                 |                                  |
| 2023-11                     | 478 (5.7)                   | 317                        | 66.3          |                 |                                  |
| 2023-12                     | 542 (6.5)                   | 354                        | 65.3          |                 |                                  |
| 2024-01                     | 490 (5.9)                   | 280                        | 57.1          |                 |                                  |
| 2024-02                     | 426 (5.1)                   | 269                        | 63.1          |                 |                                  |
| 2024-03                     | 435 (5.2)                   | 235                        | 54.0          |                 |                                  |
| 2024-04                     | 488 (5.8)                   | 286                        | 58.6          |                 |                                  |

|         |           |     |      |
|---------|-----------|-----|------|
| 2024-05 | 539 (6.4) | 327 | 60.7 |
| 2024-06 | 554 (6.6) | 347 | 62.6 |
| 2024-07 | 786 (9.4) | 470 | 59.8 |

Abbreviation: ICU, intensive care unit.

The study population was drawn from all six participating regions, with the largest contribution from South Peninsular Malaysia (23.6%), followed by Sarawak (19.7%) and North Peninsular Malaysia (16.8%). The largest age subgroup was infants aged 1–11 months (29.6%), followed by adults aged 18–64 years (25.1%) and children aged 1–4 years (20.5%). Overall positivity differed significantly across subgroup categories, with the strongest variation observed by age group ( $P < 0.001$ , Cramér's  $V = 0.285$ ) and ward ( $P < 0.001$ ,  $V = 0.242$ ). By comparison, regional variation was statistically significant but much smaller in magnitude ( $P < 0.001$ ,  $V = 0.062$ ), and time-related variation was likewise modest (year-month  $P < 0.001$ ,  $V = 0.088$ ; year  $P < 0.001$ ,  $V = 0.060$ ). Positivity was highest in the paediatric general ward (74.6%), followed by the paediatric intensive care unit (65.2%), whereas it was lower in adult wards. By sex, positivity was 64.7% in females and 61.1% in males.

**Table 2.** Overall detection frequency of respiratory pathogens

| Pathogen                                     | Detected (n) | Detected (% of all samples, $N = 8361$ ) <sup>a</sup> |
|----------------------------------------------|--------------|-------------------------------------------------------|
| Rhinovirus/enterovirus <sup>b</sup>          | 1986         | 23.8                                                  |
| Respiratory syncytial virus A/B <sup>c</sup> | 1149         | 13.7                                                  |
| Influenza A H1N1/2009                        | 403          | 4.8                                                   |
| Adenovirus                                   | 316          | 3.8                                                   |
| Human metapneumovirus A/B <sup>c</sup>       | 309          | 3.7                                                   |
| <i>Mycoplasma pneumoniae</i>                 | 296          | 3.5                                                   |
| Influenza A H3                               | 290          | 3.5                                                   |
| <i>Bordetella pertussis</i>                  | 289          | 3.5                                                   |
| SARS-CoV-2                                   | 233          | 2.8                                                   |
| Parainfluenza virus 3                        | 230          | 2.8                                                   |
| Bocavirus                                    | 186          | 2.2                                                   |
| Influenza B                                  | 179          | 2.1                                                   |
| Parainfluenza Virus 1                        | 86           | 1.0                                                   |
| Coronavirus OC43                             | 82           | 1.0                                                   |
| Parainfluenza Virus 4                        | 76           | 0.9                                                   |
| Coronavirus NL63                             | 71           | 0.8                                                   |
| Influenza A (unsubtyped)                     | 48           | 0.6                                                   |
| Coronavirus HKU1                             | 38           | 0.5                                                   |
| Parainfluenza Virus 2                        | 35           | 0.4                                                   |
| Coronavirus 229E                             | 23           | 0.3                                                   |
| Influenza A H1                               | 12           | 0.1                                                   |
| <i>Legionella pneumophila</i>                | 6            | 0.1                                                   |

a Percentages are calculated based on the total number of samples tested ( $N = 8,361$ ). Because 973 samples contained co-detections, the sum of individual pathogen detections (6,343) exceeds the total number of positive samples (5,252).

b The QIAstat-Dx Respiratory SARS-CoV-2 Panel assay detects rhinovirus and enterovirus but does not differentiate between them.

c For respiratory syncytial virus (RSV) and human metapneumovirus (hMPV), the QIAstat-Dx Respiratory SARS-CoV-2 Panel assay detects these pathogens but does not differentiate between subtypes (e.g., RSV A vs B, hMPV A vs B).

The overall pathogen profile is summarised in Table 2. Rhinovirus/enterovirus was the most frequently detected pathogen, identified in 1,986 samples (23.8%), followed by RSV A/B in 1,149 (13.7%) and influenza A H1N1/2009 in 403 (4.8%). Other common pathogens were adenovirus (3.8%), human metapneumovirus A/B (3.7%), *Mycoplasma pneumoniae* (3.5%), influenza A H3 (3.5%), and *Bordetella pertussis* (3.5%). Thus, the pathogen landscape was dominated by viruses, with atypical bacterial detections contributing a much smaller proportion of the total burden.

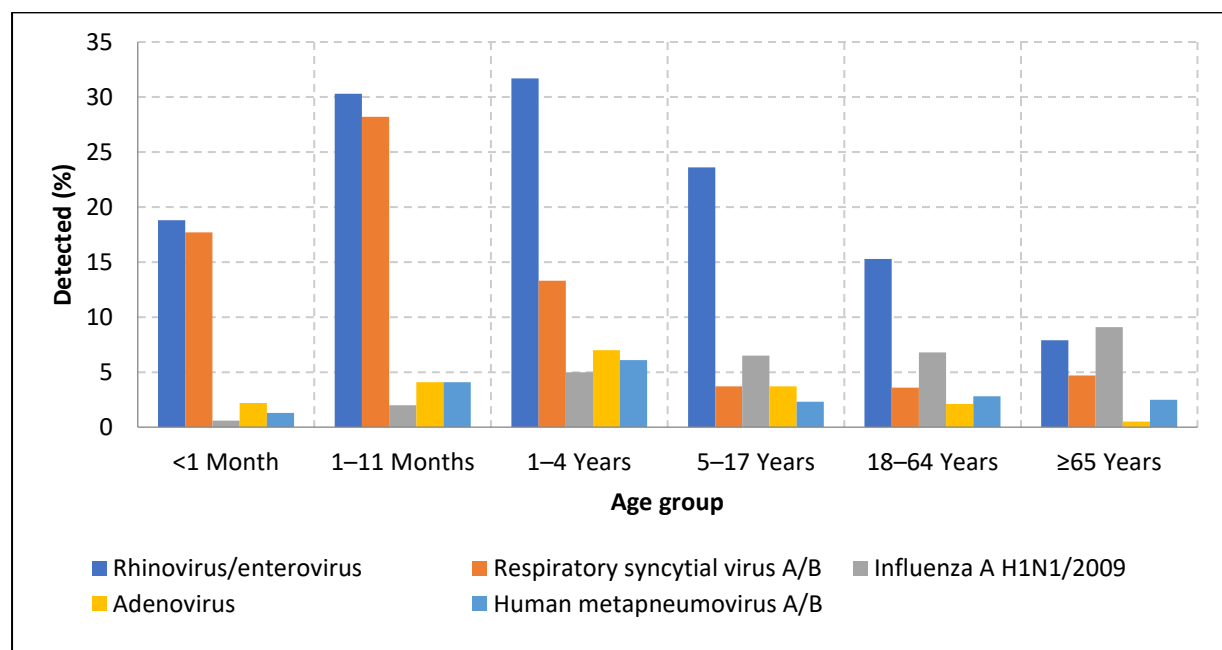


**Figure 2.** Distribution of the top 5 pathogens by age group

The age patterns of the five leading pathogens are shown in Figure 2. Rhinovirus/enterovirus was the most frequently detected pathogen in every age group; its relative predominance was greatest in children aged 1–4 years (31.7%) and infants aged 1–11 months (30.3%), then declined steadily with advancing age to 7.9% in older adults. In contrast, influenza A H1N1/2009 showed the opposite pattern, rising from 0.6% in neonates to 9.1% in adults aged ≥65 years. RSV showed a distinct biphasic age distribution, with a primary peak in infants aged 1–11 months (28.2%), a lower but still notable rate in neonates (17.7%), and a smaller secondary increase in older adults (4.7%) after falling to low levels in older children and adults. Adenovirus and human metapneumovirus, like rhinovirus/enterovirus, were concentrated in childhood and both peaked in children aged 1–4 years (7.0% and 6.1%, respectively). All five pathogens differed significantly by age group (all  $P < 0.001$ ). The strongest age associations were seen for RSV ( $V = 0.300$ ), followed by rhinovirus/enterovirus ( $V = 0.186$ ) and influenza A H1N1/2009 ( $V = 0.114$ ); by comparison, the corresponding effects were smaller for adenovirus ( $V = 0.102$ ) and human metapneumovirus ( $V = 0.076$ ).

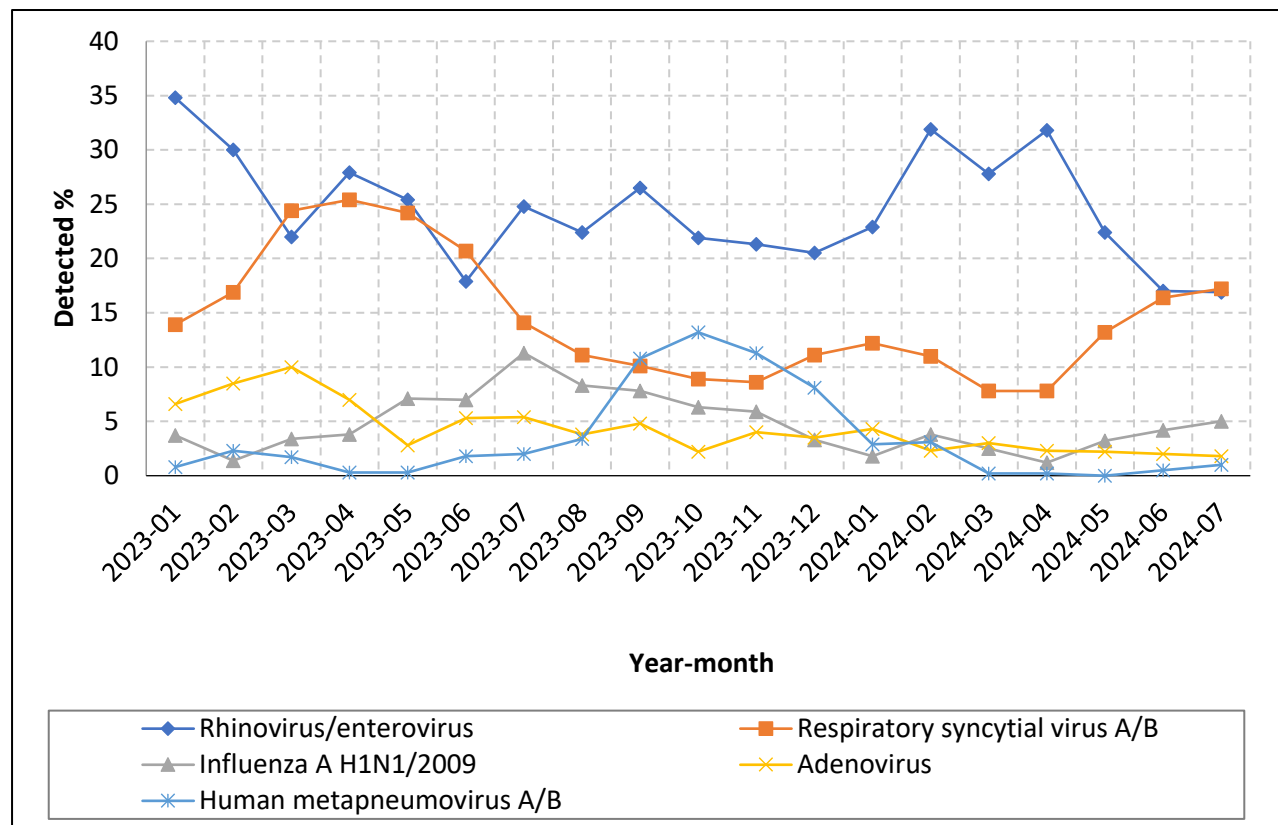


**Figure 3.** Monthly detection rates of the top 5 pathogens

Temporal trends are illustrated in Figure 3 and summarised in the quarterly analyses. Quarterly positivity peaked in 2023 Q1 (69.7%), then remained relatively stable through the rest of 2023 (64.1%–65.6%), before falling in 2024 Q1 (58.0%) and showing only partial recovery in 2024 Q2 (60.7%) and 2024 Q3 (59.8%). At the pathogen level, the five leading pathogens showed clearly distinct temporal profiles. Rhinovirus/enterovirus circulated throughout the study period, peaking in January 2023 (34.8%), with secondary peaks in February and April 2024 (31.9% and 31.8%, respectively). RSV showed the sharpest, most concentrated peak, rising through early 2023 to a maximum in April 2023 (25.4%), then declining before a smaller resurgence in mid-2024. Influenza A H1N1/2009 peaked later, with its highest monthly detection in July 2023 (11.3%), consistent with a mid-year rise rather than the RSV pattern. Adenovirus peaked in March 2023 (10.0%), while human metapneumovirus A/B peaked latest, increasing sharply in late 2023 and reaching its highest monthly rate in October 2023 (13.2%). Taken together, these findings show that the main respiratory pathogens did not move in parallel over time but followed pathogen-specific temporal trajectories.

Overall, the analysis demonstrated six main patterns. First, the respiratory multiplex PCR positivity rate was high at 62.8%. Second, co-detections were observed in a substantial proportion of samples, occurring in 11.6% of all samples and 18.5% of positive samples. Third, the pathogen profile was dominated by viral detections. Fourth, rhinovirus/enterovirus was the leading pathogen overall, followed by RSV and influenza A H1N1/2009. Fifth, age-related variation was more pronounced than regional variation. Sixth, the major pathogens showed distinct temporal patterns, with rhinovirus/enterovirus demonstrating persistent circulation, RSV peaking in April 2023, adenovirus peaking in March 2023, influenza A H1N1/2009 showing a mid-year increase, and human metapneumovirus A/B peaking later in October 2023.

## DISCUSSION

This multicentre study describes the prevalence, distribution, co-detection patterns, demographic associations, and temporal trends of respiratory pathogens detected by the QIAstat-Dx Respiratory SARS-CoV-2 Panel in Malaysian public hospitals. The findings showed a high overall positivity rate, predominance of viral pathogens, rhinovirus/enterovirus as the leading pathogen, substantial co-detection, marked age-related variation, and distinct temporal patterns among the major pathogens.

The overall positivity rate of 62.8% indicates substantial diagnostic yield of respiratory multiplex PCR in routine hospital practice. Reported positivity varies across studies according to patient population, pathogen spectrum, and assay interpretation. In critically ill immunocompromised adults, Contier et al. reported a positivity rate of 29.8% using a pneumonia-focused panel, whereas paediatric studies have shown considerably higher positivity [6]. Popova et al. reported 100% positivity in hospitalised children tested using the Allplex Respiratory Full Panel with a positive cycle threshold (Ct) cutoff of <42, decreasing to 75% when a stricter cutoff of <28 was applied [7]. Tai et al. similarly found an overall positivity rate of 84.2% in paediatric patients tested using a FilmArray multiplex PCR panel [8]. These comparisons suggest that the positivity observed in the present study is substantial, while also highlighting the importance of case mix and analytic thresholds when comparing multiplex PCR studies.

Co-detection was observed in 11.6% of all samples and 18.5% of positive samples, confirming that multiple-pathogen detection is a relevant feature of multiplex respiratory testing. This rate was lower than that reported in a paediatric cohort studied by Popova et al., where co-detection ranged from 22.4% with a stricter positive Ct cutoff of <28 to 82.9% with a positive Ct cutoff of <42 [7]. These differences again suggest that co-detection estimates are strongly influenced by age distribution, assay design, and reporting thresholds. Even so, the present findings indicate that co-detection is common enough to remain clinically important in routine hospital practice.

The predominance of viral pathogens in this study is consistent with previous respiratory multiplex PCR and surveillance studies. In Southern Brazil, Ruivo et al. found that expanded molecular testing identified at least one respiratory pathogen in 43.5% of tested severe acute respiratory infection cases, with human rhinovirus being the most frequently detected virus (63.3% of positive cases) [9]. Norton et al. similarly reported that among symptomatic patients negative for SARS-CoV-2, influenza, and RSV, 31% had another pathogen detected on extended panel testing, with rhinovirus/enterovirus accounting for 60% of positive findings [10]. In Malaysia, Low et al. reported that rhinovirus/enterovirus was also the most prevalent pathogen among paediatric patients with acute respiratory illness (29.7%), followed by influenza virus and RSV [11]. Yew et al. also identified rhinovirus/enterovirus and RSV as the two predominant viruses among Malaysian children aged five years and younger, followed by adenovirus, human bocavirus, and human metapneumovirus [12]. Together, these studies support the present finding that respiratory pathogen detections are predominantly viral and that rhinovirus/enterovirus is a leading pathogen across diverse clinical settings, particularly among children.

A small proportion of samples showed unusual patterns of influenza A detection. Forty-eight samples (0.6%) tested positive for influenza A without detection of the H1, H3, or H1N1/2009 subtypes. Among cases with recorded Ct values, the median influenza A Ct was 34.7, with most values above 30, suggesting that low target concentration may explain many of these unsubtyped detections. The QIAstat-Dx panel employs a generic influenza A target together with subtype-specific targets; influenza A-positive/subtype-negative results may therefore also occur with genetically divergent or zoonotic

influenza A viruses [13]. An additional 12 samples (0.14%) were recorded as positive for the seasonal influenza A H1 target. This was unexpected because the pre-2009 seasonal H1N1 lineage was displaced following the emergence of A(H1N1)pdm09, and currently circulating human influenza A(H1N1) viruses are descendants of the 2009 pandemic lineage [14]. Importantly, the QIAstat-Dx panel retains a separate seasonal H1 target, despite the historical seasonal H1 lineage no longer being considered in circulation, partly to enable discrimination of unusual H1 genetic patterns, including potentially zoonotic strains [13]. Both the unsubtype influenza A and H1-target detections were sporadically distributed throughout the study period without sustained temporal clustering. In the absence of repeat testing, epidemiological exposure information, reference-laboratory confirmation, or sequencing, these findings should not be interpreted as evidence of novel influenza infection or re-emergence of the historical seasonal H1 lineage. Nevertheless, persistent or epidemiologically unusual influenza A-positive/subtype-negative or unexpected H1-target results warrant further investigation and confirmatory molecular characterisation.

The marked age-related variation observed in this study is consistent with previous reports showing that respiratory viruses have distinct age-specific epidemiologic patterns. This study found that age-group differences were not only statistically significant but also moderate in magnitude overall (Cramér's  $V = 0.285$ ), with the strongest pathogen-specific age associations seen for RSV ( $V = 0.300$ ), followed by rhinovirus/enterovirus ( $V = 0.186$ ) and Influenza A H1N1/2009 ( $V = 0.114$ ). By comparison, the effects were smaller for adenovirus ( $V = 0.102$ ) and human metapneumovirus A/B ( $V = 0.076$ ). In Kyoto, Matsumura et al. found significant differences in virus detection across age groups, with rhinovirus/enterovirus and RSV predominating in younger children, whereas influenza viruses and seasonal coronaviruses were more prominent in older children and adults [15]. This pattern is also supported by broader RSV literature, which recognises RSV as a major cause of acute lower respiratory infection, particularly in infants and young children, while also contributing importantly to disease burden in older adults [16]. In contrast, influenza-related hospitalisation has been shown to shift toward older age groups and those with underlying illness; Fu et al. reported that influenza B-related viral pneumonia mainly affected older adults and patients with comorbidities, whereas human influenza A pneumonia was relatively more frequent in younger adults [17]. Paediatric data from Suzhou similarly showed that RSV and rhinovirus predominated in children aged under 3 years, while pathogen distribution changed with increasing age [18]. Human metapneumovirus also appears to follow an age-related pattern, with greatest clinical relevance in young children, older adults, and immunocompromised individuals [19]. Taken together, these studies reinforce the present finding that age was one of the strongest epidemiologic discriminators in the dataset.

In the present study, the leading pathogens showed distinct temporal patterns, which were compared with previous Malaysian studies. Rhinovirus/enterovirus circulated throughout the study period, with the highest detection in January 2023 and secondary peaks in February and April 2024. Local findings have varied across observation periods: Yew et al. reported pronounced mid-year rhinovirus/enterovirus activity between April and July 2015 [12], whereas a five-year study by Low et al. (2015–2019) found rhinovirus/enterovirus detected year-round without a consistent seasonal pattern [11]. In the current study, RSV showed the sharpest peak in April 2023, followed by a smaller resurgence in mid-2024. Earlier Malaysian studies likewise demonstrated recurrent but variable RSV peaks. Chan et al. reported an annual peak of RSV infection from November to January among young children hospitalised in Kuala Lumpur between 1982 and 1997 [20]. Khor et al., in a 27-year study conducted in Kuala Lumpur (1982–2008), similarly found year-round respiratory virus circulation, with RSV activity predominantly peaking from September to December [21]. A later study by Chan et al. (2017–2021) identified periods of increased RSV activity during July–August and October–December in Central Peninsular Malaysia [22]. These differences suggest that RSV activity in Malaysia is recurrent

but does not follow a single fixed seasonal peak nationwide; rather, it varies across years, locations, and patient populations.

In contrast to the earlier peaks of rhinovirus/enterovirus and RSV, influenza A H1N1/2009 showed its highest monthly detection in July 2023. This falls within one of the periods of increased influenza activity reported by Sam et al. in laboratory-based Malaysian surveillance data from 2011 to 2016, which showed year-round circulation with variable increases during November–December, January–March, and July–September [23]. Adenovirus showed a different pattern, peaking in March 2023. This differs from the mid-year adenovirus peak reported by Yew et al. during 2014–2015 [12], but is consistent with the later study by Low et al., which found no consistent seasonal variation in adenovirus between 2015 and 2019 [11]. Human metapneumovirus A/B showed the latest peak in the present study, reaching its maximum in October 2023. Malaysian data on hMPV are also heterogeneous: Low et al. reported year-round detection without a consistent seasonal pattern [11], whereas Chow et al. observed increased hMPV activity during the northeast monsoon period from November to April in Kuala Lumpur, with higher prevalence associated with greater relative humidity and more rainy days [24]. The October 2023 increase observed here therefore occurred close to the period of increased activity described by Chow et al., although the 19-month duration of the present study is insufficient to establish recurring seasonality. Overall, the Malaysian literature and the present findings suggest that respiratory pathogens demonstrate distinct and sometimes variable temporal patterns, supporting pathogen-specific surveillance rather than reliance on overall respiratory positivity alone.

This study has several limitations. First, this was a retrospective laboratory-based analysis and therefore represents only patients selected for testing rather than the underlying population burden of disease. Second, clinical information was not available, so symptom severity, comorbidities, vaccination status, antimicrobial use, and outcomes could not be assessed. Third, detection of a respiratory target by multiplex PCR does not necessarily establish causation, particularly for rhinovirus/enterovirus, for which prolonged PCR detection has been reported in approximately one-third of infections [25], and in samples with multiple viral detections, whose clinical significance remains uncertain and requires correlation with the clinical presentation [26]. Fourth, site-level differences in test-ordering practices and case mix may have influenced the observed distributions. Fifth, the final quarter of the study period included July 2024 only, so comparisons involving that quarter should be interpreted cautiously. Finally, the final analytical dataset excluded records with incomplete demographic information, and although the dataset underwent cleaning and review, routine multicentre laboratory data remain vulnerable to minor coding inconsistencies.

Despite these limitations, the study provides useful real-world epidemiologic insight into respiratory pathogen detection in Malaysian public hospitals. The findings indicate that respiratory multiplex PCR results in this setting are characterised by a high positivity rate, a meaningful burden of co-detection, predominance of viral pathogens, and age-related differences that are substantially stronger than regional variation. In particular, RSV showed the strongest age association among the leading pathogens, while rhinovirus/enterovirus remained the most frequently detected pathogen across all age groups. The major pathogens also followed distinct temporal trajectories rather than moving in parallel over time, supporting the value of pathogen-specific surveillance for diagnostic interpretation, hospital preparedness, and future respiratory surveillance efforts.

## CONCLUSION

In conclusion, this multicentre analysis of 8,361 respiratory multiplex PCR results from Malaysian public hospitals showed a high pathogen detection rate, with 62.8% of samples yielding at least one respiratory target. Respiratory pathogen detections were predominantly viral, with rhinovirus/enterovirus as the most frequently detected pathogen overall, followed by RSV A/B and Influenza A H1N1/2009. Co-detections were also observed in a meaningful proportion of samples, occurring in 11.6% of all samples and 18.5% of positive samples. Marked age-related differences and distinct pathogen-specific temporal patterns were also observed.

These findings support the potential role of multiplex respiratory PCR within a tiered diagnostic and surveillance strategy, particularly in tertiary hospitals, intensive care, and paediatric settings. The observed temporal patterns may assist with laboratory preparedness, antiviral stock management, infection-control planning, and influenza vaccination strategies; however, the 19-month study period was insufficient to establish stable national seasonality, and the findings should complement existing national surveillance data.

The platform may prove valuable for early outbreak recognition, facilitating patient isolation or cohorting strategies, antimicrobial stewardship, and hospital resource planning. Nevertheless, routine nationwide implementation should be guided by laboratory capacity, turnaround time, cost-effectiveness and evidence of clinical benefit, particularly for pathogens without specific treatment. Further studies linking molecular results with clinical outcomes and healthcare utilisation are required before broader systematic implementation can be recommended.

**Supplementary Materials:** Not applicable

**Data Availability:** The data supporting the findings of this study are not publicly available because they were derived from routine laboratory records from participating Malaysian public hospitals and may be subject to institutional and ethical restrictions. De-identified data may be available from the corresponding author upon reasonable request and subject to approval by the relevant authorities.

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