

Positivity rate of respiratory syncytial virus (RSV) using multiplex PCR test in Indonesia

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Abstract

Introduction: Respiratory syncytial virus (RSV) is highly prevalent as a cause of acute respiratory tract infection. Detection of RSV using nucleic acid amplification tests provides rapid results that can help physicians manage patients with acute respiratory symptoms and avoid unnecessary antibiotics. This study aimed to report the positivity rate of RSV among patients with acute respiratory symptoms.

Methodology: Nasopharyngeal swab specimens were obtained from primary healthcare facilities and hospital outpatient clinics from January 2023 to June 2024. The specimens were processed using the multiplex QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN GmbH, Hilden, Germany).

Results: A total of 1339 tests were performed and RSV was positive in 10.3% of tested patients (138/1339). Regarding the monthly positivity rate, the highest rate of RSV positivity was in November 2023, followed by January 2023, and March 2023.

Conclusions: This study shows a moderate RSV positivity rate in outpatient settings and suggests a seasonal trend, with higher circulation during the rainy season. These findings highlight the value of RSV surveillance for guiding clinical and public health responses in tropical regions.

Key words: respiratory syncytial virus; RSV; PCR; multiplex PCR; QIAstat-Dx; Indonesia.

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Introduction

Respiratory syncytial virus (RSV) is an RNA virus belonging to the Paramyxoviridae family, which predominantly affects the respiratory system [1]. It is a major cause of bronchiolitis and pneumonia, particularly in infants, elderly individuals, and those with compromised immune systems [2]. The annual RSV-attributable hospitalization rates for individuals 75 years and older, ranges from 110.9 per 100,000 population for respiratory hospitalizations to 1199.8 per 100,000 for cardiorespiratory hospitalizations [3]. In Indonesia, an average of 43 cases were reported weekly, reflecting the virus's seasonal nature—typically peaking during winter and autumn in temperate regions and during the rainy season in tropical countries [4].

The detection and identification of multiple viral pathogens is crucial for timely and accurate diagnosis in clinical settings [5]. Traditional methods, including single-pathogen assays, are often time-consuming and inefficient when multiple viruses may be responsible for similar symptoms [6]. Multiplex systems, designed to simultaneously detect multiple viruses in a single test, address this gap [7]. These systems leverage molecular techniques, most commonly polymerase chain reaction (PCR), to amplify nucleic acid sequences of multiple pathogens in one reaction, streamlining

diagnosis and improving patient management [8].

The QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN GmbH, Hilden, Germany) is a multiplexed real-time PCR diagnostic tool designed to detect and differentiate a wide range of respiratory pathogens, including RSV [9]. The panel is an advanced tool that rapidly identifies the presence of multiple respiratory viruses and bacteria from a single patient sample, such as a nasopharyngeal swab [10]. The QIAstat-Dx cartridge allows the loading of the nasopharyngeal swab resuspended in transport medium through the liquid port or the direct insertion of the nasopharyngeal swab into the cartridge without additional manipulation [11]. This system allows sample testing on demand in any laboratory, even in a non-PCR-trained laboratory, or as a point-of-care (PoC) test with minimal training [12]. There is a lack of multi-year RSV prevalence data across all age groups in Indonesia using multiplex in primary and outpatient care. This study aimed to report the positivity rate of RSV using multiplex PCR, in a period of two years, across all age groups in Indonesia, as well as coinfection of RSV with other viruses or atypical bacteria.

Methodology

This was a retrospective descriptive study of

outpatients at one primary healthcare facility and three hospitals in Jakarta, Indonesia, conducted from January 2023 to June 2024. The inclusion criteria were patients of all ages presenting with acute respiratory infection (ARI), including symptoms such as cough, runny nose, shortness of breath, sore throat, and fever lasting less than 7 days. The exclusion criterion was patients who did not exhibit clinical manifestations consistent with acute respiratory infection. This study was granted ethical approval from the Institutional Review Board (IRB) of Persahabatan Hospital, Jakarta, Indonesia (Number 0232/KEPK-RSUPP/10/2024).

Each patient provided a single sample, and sample collection was performed using consecutive sampling. Nasopharyngeal swab techniques, sample collection, handling procedures of transport, cartridge loading, sample preparation, and internal control were performed according to the 'QIAstat-Dx® Respiratory SARS-CoV-2 Panel Instructions for Use' Handbook.

This multiplex panel detects multiple respiratory pathogens, including influenza B, parainfluenza virus 1–4, coronaviruses (OC43, NL63, HKU1, 229E), SARS-CoV-2, rhinovirus/enterovirus, respiratory syncytial virus A + B, *Mycoplasma pneumoniae*, human metapneumovirus A + B, *Bordetella pertussis*, bocavirus, and adenovirus.

Positive or negative results were determined based on the cycle threshold (Ct) value for each pathogen, with a Ct cutoff of 40. Specimens with a Ct value ≤ 40 were reported as “detected” (positive), whereas specimens with a Ct value > 40 were reported as “not detected” (negative). All samples were processed in the

same laboratory (Diagnos Laboratorium, Jakarta, Indonesia) to ensure consistency and reliability of results. The distribution of RSV-positive cases was divided into quarters: Q1, from January to March; Q2, from April to June; Q3, from July to September; and, Q4, from October to December.

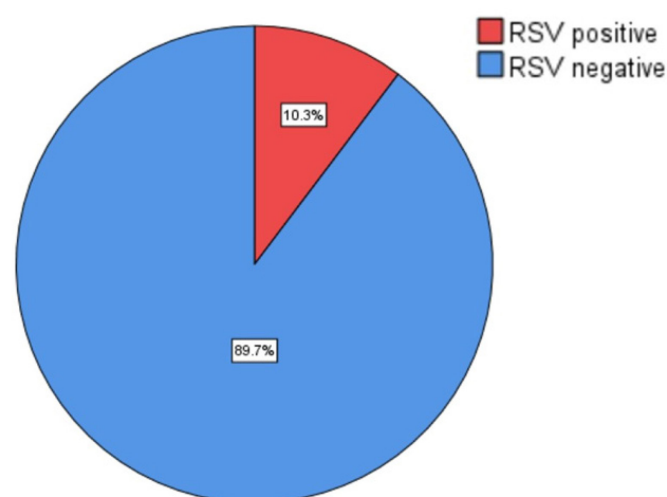
Data were entered into Microsoft Excel and analyzed using SPSS version 27. Categorical variables were summarized as frequencies and percentages, while continuous variables were assessed for normality using the Kolmogorov–Smirnov test. Normally distributed data ($p > 0.05$) were reported as mean $\pm$ standard deviation (SD); non-normally distributed data were expressed as median with minimum and maximum values. The RSV positivity rate was calculated as the proportion of positive cases among all samples, with 95% confidence intervals (CIs) estimated using the Wald method. Categorical variables were compared using the Chi-square or Fisher's exact test for bivariate analysis. Variables with a p value < 0.25 were selected for further analysis, and odds ratios (ORs) with 95% CIs were calculated to assess associations. A p value < 0.05 was considered statistically significant.

Results

A total of 1,339 participants were analyzed in this study during the period from January 2023 to June 2024 (Table 1). The participants' median age was 6 years. The majority of participants belonged to the 0–9 years age group, were female, and received care in outpatient services. The multiplex PCR indicated that 10.3% of participants tested positive for RSV (Figure 1).

Next, the monthly RSV positive cases and positivity rates from January 2023 to June 2024 were analyzed (Figure 2). The number of RSV-positive cases and positivity rates fluctuated throughout the observation period, with a noticeable increase at the end of 2023 and

Figure 1. Proportion of respiratory syncytial virus (RSV) positivity among multiplex PCR-tested patients.



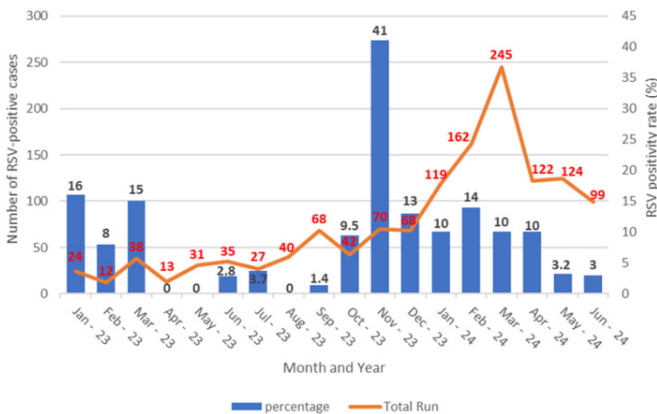
RSV positivity rate among tested patients. 95% confidence interval: 8.7%–11.9%.

Table 1. Sociodemographic characteristics of participants^a.

Variable	N = 1339 (%)
Age (years) (mean/median)	6 (0.5–92)
Age group (years)	
0–9	818 (60.9)
10–17	106 (7.9)
18–59	362 (27)
> 60	53 (4.2)
Gender	
Male	752 (56.1)
Female	587 (43.9)
Patient care setting	
Inpatient services	291 (21.7)
Outpatient services	857 (64)
Emergency services	191 (14.3)
Respiratory syncytial virus (RSV) A and B test results	
Positive	138 (10.3)
Negative	1201 (89.7)

^aUnivariate analysis.

Figure 2. Comparison of total tests and positive cases per month.

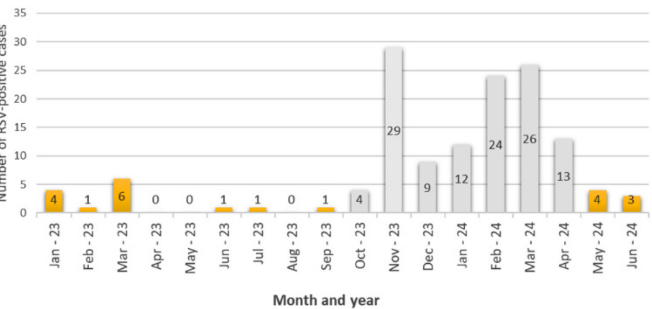


the beginning of 2024. The highest number of RSV-positive cases (29 cases) and the highest positivity rate (41.4%) was recorded in November 2023. In contrast, the lowest values for both parameters were observed in April, May, and August 2023. Overall, the data indicated a seasonal pattern of RSV infection, with higher activity during the rainy season or late in the year and lower activity in the middle of the year (Figure 3).

The distribution of RSV-positive cases varied significantly across quarters (Table 2). Compared to Q1, the odds of RSV positivity were significantly lower during Q2 (OR = 0.1, 95% CI: 0.01–0.58; $p = 0.013$) and Q3 (OR = 0.1, 95% CI: 0.01–0.42; $p = 0.001$), indicating a substantial decrease in RSV activity during the middle of the observation period. Although the odds appeared higher in Q4 (OR = 1.7, 95% CI: 0.84–3.60; $p = 0.134$), the difference was not statistically significant. RSV positivity again decreased significantly in Q6 (OR = 0.3, 95% CI: 0.16–0.77; $p = 0.012$). Thus, notable reductions were seen in Q2, Q3, and Q6 compared to Q1.

Coinfections among RSV-positive cases were observed only between October 2023 and March 2024, with no coinfections detected during other months in the surveillance period of January 2023 to June 2024 (Figure 4). A single coinfection was reported with adenovirus, human metapneumovirus A + B, influenza

Figure 3. Trend of respiratory syncytial virus (RSV)-positive cases by month among tested patients (n = 1339).



Gray area indicates the rainy season, and yellow area represents the dry season.

A+ H1N1 pdm09, *Bordetella pertussis*, bocavirus, and rhinovirus/enterovirus. The peak of RSV coinfections occurred in February 2024. Rhinovirus/enterovirus remained the most frequent coinfecting pathogen throughout the study (Table 3).

Figure 4. Distribution of coinfecting viruses among respiratory syncytial virus (RSV)-positive cases by month.

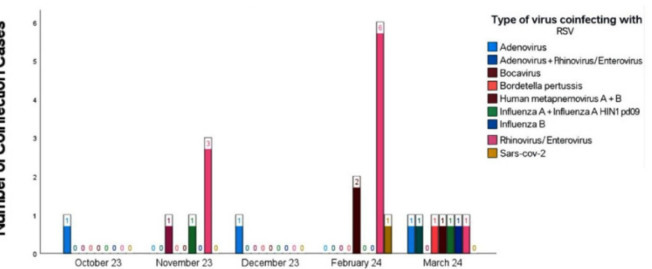


Table 2. Distribution of respiratory syncytial virus (RSV) A and B test results by quarter with statistical comparison and 95% confidence intervals.

Variable	RSV A and B test results			p value	OR (95% CI)
	Positive n (%)	Negative n (%)	Total		
Quarter					
Q1	11(14.8)	63 (85.2)	74		ref
Q2	1 (1.2)	78 (98.8)	79	0.013	0.1 (0.01–0.58)
Q3	2 (1.4)	133 (98.6)	135	0.001	0.1 (0.01–0.42)
Q4	42 (23.3)	138 (76.7)	180	0.134	1.7 (0.84–3.60)
Q5	62 (11.7)	464 (88.3)	526	0.449	0.7 (0.28–1.5)
Q6	20 (5.7)	325 (94.3)	345	0.012	0.3 (0.16–0.77)

Table 3. Summary of coinfections in respiratory syncytial virus (RSV) patients.

Coinfecting virus	Number of coinfection cases (n)	Percentage of RSV-positive cases (%)	Percentage of total tests (%)
Respiratory syncytial virus A + B*	115	83.3	8.6
Rhinovirus/Enterovirus	10	7.2	0.7
Adenovirus	3	2.2	0.2
Human metapneumovirus A + B	3	2.2	0.2
Influenza A+ Influenza A H1N1 pdm09**	2	1.4	0.1
Influenza B	1	0.7	0.1
SARS-CoV-2	1	0.7	0.1
<i>Bordetella pertussis</i>	1	0.7	0.1
Bocavirus	1	0.7	0.1
Adenovirus + Rhinovirus/Enterovirus**	1	0.7	0.1
Parainfluenza virus 1	0	0	0
Parainfluenza virus 2	0	0	0
Coronavirus OC43	0	0	0
Coronavirus NL63	0	0	0
Coronavirus HKU1	0	0	0
Coronavirus 229E	0	0	0
<i>Chlamydomphila pneumoniae</i>	0	0	0
Parainfluenza virus 3	0	0	0
Parainfluenza virus 4	0	0	0
<i>Mycoplasma pneumoniae</i>	0	0	0
Influenza HKU1	0	0	0
Influenza A H1N1 pdm09	0	0	0
Influenza A	0	0	0

*Mono-infection; **There was co-infection with more than two viruses.

Finally, the association of patient characteristics with the RSV positivity was analyzed. Age was significantly associated with RSV positivity ($p = 0.011$), as summarized in Table 4. Patients aged 0–9 years were 4.2 times more likely to test positive for RSV (OR = 4.2, 95% CI: 2.39–7.47; $p = 0.001$) compared to patients aged 18–59 years. No significant associations were observed for the 10–17 years and ≥ 60 years age groups when compared to the 18–59 years reference group. RSV positivity did not differ significantly by gender ($p = 0.538$). Outpatient cases had significantly lower odds of RSV positivity compared to emergency department cases (OR = 0.4, 95% CI: 0.28–0.70; $p = 0.001$), with no significant difference for inpatients ($p = 0.331$).

Discussion

A total of 1,339 nasopharyngeal swab samples were analyzed between January 2023 and June 2024 using the multiplex QIAstat-Dx respiratory SARS-CoV-2 panel. Among these, 138 samples tested positive for RSV, yielding a positivity rate of 10.3% (95% CI: 8.7%–11.9%). The positivity rate of RSV was reported to be 15.9% (3652/ 23000) in Malaysia [13], 5.8% (487/8436) in Singapore [14], and 11.8% (122/1036) in the Philippines [15]. A previous RSV study in Indonesia involved 2,677 children hospitalized with severe lower respiratory illness (LRI) and revealed a 23% positivity rate [16]. A study conducted in Bandung, Indonesia, reported that 7.9% of subjects with LRI were RSV-positive [17]. The findings of this study are consistent with global reports, which identified RSV

Table 4. Association between demographic and clinical characteristics, and respiratory syncytial virus (RSV) positivity among patients who underwent RSV testing.

Variable	RSV A and B test results			Total	p value	OR (95% CI)
	Mean/Median	Positive	Negative			
Age (years) ^a	6 (0.5–92)				0.011*	
Age groups (years) ^b						
0–9		120	698	818	0.001*	4.2 (2.39–7.47)
10–17		2	104	106	0.334	0.4 (0.11–2.13)
18–59		14	348	362		Ref
> 60		2	51	53	0.973	0.9 (0.21–4.41)
Gender						
Male		74	678	752	0.538	0.8 (0.61–1.25)
Female		64	523	587		
Patient care setting						
Outpatient services		68	789	857	0.001*	0.4 (0.28–0.70)
Inpatient services		39	252	291	0.331	0.7 (0.46–1.2)
Emergency services		31	160	191		ref

Ref: reference category; OR: odds ratio; CI: confidence interval; * $p < 0.05$ was considered statistically significant; ^aAge is presented as median (min–max); ^bChi-square or Fisher's exact test was used for categorical variables.

in 9.7% (95% CI: 8.7–10.7) of influenza-like illness episodes among 6,266 children worldwide [18]. Although the RSV positivity rate in this study (10.3%) is comparable to those reported in Malaysia, Singapore, and the Philippines; direct comparisons should be interpreted cautiously due to differences in study populations, age groups, and healthcare settings. For example, some studies were conducted on pediatric inpatients, while the present study included all age groups in outpatient settings.

The monthly distribution of RSV-positive cases demonstrated a clear seasonal pattern. The number of cases began to rise in November 2023 (29 cases) following a low period between April and September 2023 (0–1 case per month). The highest peaks were observed in February (24 cases) and March 2024 (26 cases), followed by a gradual decline until June 2024 (3 cases). This pattern indicates that RSV transmission peaked during the late and early months of the year, coinciding with Indonesia's rainy season, a period commonly associated with an increased incidence of respiratory infections. Similarly, a study conducted in Bogor, Indonesia, from 2017 to 2019 reported that pneumonia incidence among children under 5 years old increased during the transitional season, particularly between September and November, when relative humidity reached approximately 80% [18].

Coinfections were identified in a subset of RSV-positive patients, with the most frequent being RSV and rhinovirus/enterovirus (7.2%), followed by adenovirus (2.2%). Other coinfections such as influenza A (including H1N1pdm09), influenza B, SARS-CoV-2, *Bordetella pertussis*, bocavirus, human metapneumovirus A + B, and adenovirus combined with rhinovirus/enterovirus were detected less frequently (0.7% each). These findings indicate that RSV coinfection most commonly occurs with rhinovirus and, to a lesser extent, with adenovirus and influenza A. Although coinfections are generally thought to cause more severe clinical manifestations than single infections, this study did not provide clinical symptom data [19]. In contrast, a global systematic review found no significant difference in clinical severity between RSV mono-infections and RSV coinfections, except for RSV–human metapneumovirus (hMPV) coinfections, which were associated with a higher likelihood of requiring intensive care [20]. Co-infection with RSV and human metapneumovirus can lead to more severe illness than RSV alone, especially in young children, increasing risks for longer hospital stays and more severe hypoxia [21].

This study has several strengths, including a large

sample size (1,339 patients) and the use of the multiplex QIAStat-Dx respiratory SARS-CoV-2 panel, which enables rapid, sensitive, and specific detection of RSV and other respiratory viruses [22]. The study also demonstrated the seasonal distribution of RSV and identified coinfections, providing a more comprehensive understanding of its epidemiology. Using real-time PCR, the panel delivers results within approximately one hour, allowing timely diagnosis that can inform treatment and isolation decisions during peak respiratory illness seasons.

The system generates an easy-to-read report, facilitating faster clinical decision-making and reducing the need for multiple separate tests. In addition, the inclusion of patients from various healthcare settings (outpatient, inpatient, and emergency services) reflects the real-world spectrum of ARI cases in the community.

However, this study has several limitations, including limited clinical data, which hinders assessment of disease severity; a descriptive design that does not allow analysis of risk factors or causal relationships; uneven patient distribution with a predominance of outpatients; limited geographic coverage; and coinfection analysis that did not evaluate clinical impact in depth. The study excluded patients who did not exhibit clinical manifestations consistent with ARI which may result in a significant bias by enriching the study population for the target disease.

Conclusions

In this preliminary descriptive study, RSV was detected in 10.3% of 1,339 patients tested for acute respiratory symptoms. The monthly analysis showed the highest positivity in November 2023, followed by March 2024 and February 2024, indicating a possible seasonal trend. These findings suggest a moderate prevalence of RSV infection among tested individuals, with an apparent increase during the rainy season typical of tropical regions. Timely detection of RSV, especially in outpatient and pediatric populations, plays a key role in improving patient management, limiting transmission, and optimizing resource allocation during seasonal surges.

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Conflict of interest

No conflict of interest is declared.

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